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     (FILE 'HOME' ENTERED AT 14:13:20 ON 19 SEP 2006)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 14:13:50 ON 19 SEP 2006
L1         27165 S THIOREDOXIN
L2          1360 S (TRUNCATED OR MODIFIED) AND L1
L3       1932122 S STAIN? OR DYE?
L4            61 S L2 AND L3
L5             2 S (INCLUSION (W)BOD? ) AND L4
L6            27 DUP REM L4 (34 DUPLICATES REMOVED)
                 E LONGO M/AU
L7           724 S E3
                 E FLYNN E/AU
L8           289 S E3
L9          1012 S L7 OR L8
L10            2 S L1 AND L9
L11            2 DUP REM L10 (0 DUPLICATES REMOVED)
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 NEWS  5  MAY 11  KOREAPAT updates resume
 NEWS  6  MAY 19  Derwent World Patents Index to be reloaded and enhanced
 NEWS  7  MAY 30  IPC 8 Rolled-up Core codes added to CA/CAplus and
                  USPATFULL/USPAT2
 NEWS  8  MAY 30  The F-Term thesaurus is now available in CA/CAplus
 NEWS  9  JUN 02  The first reclassification of IPC codes now complete in
                  INPADOC
 NEWS 10  JUN 26  TULSA/TULSA2 reloaded and enhanced with new search and
                  and display fields
 NEWS 11  JUN 28  Price changes in full-text patent databases EPFULL and PCTFULL
 NEWS 12  JUl 11  CHEMSAFE reloaded and enhanced
 NEWS 13  JUl 14  FSTA enhanced with Japanese patents
 NEWS 14  JUl 19  Coverage of Research Disclosure reinstated in DWPI
 NEWS 15  AUG 09  INSPEC enhanced with 1898-1968 archive
 NEWS 16  AUG 28  ADISCTI Reloaded and Enhanced
 NEWS 17  AUG 30  CA(SM)/CAplus(SM) Austrian patent law changes
 NEWS 18  SEP 11  CA/CAplus enhanced with more pre-1907 records

 NEWS EXPRESS  JUNE 30 CURRENT WINDOWS VERSION IS V8.01b, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 26 JUNE 2006.

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FILE 'HOME' ENTERED AT 14:13:20 ON 19 SEP 2006

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                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21
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FILE 'MEDLINE' ENTERED AT 14:13:50 ON 19 SEP 2006

FILE 'EMBASE' ENTERED AT 14:13:50 ON 19 SEP 2006

FILE 'BIOSIS' ENTERED AT 14:13:50 ON 19 SEP 2006

FILE 'BIOTECHDS' ENTERED AT 14:13:50 ON 19 SEP 2006

FILE 'SCISEARCH' ENTERED AT 14:13:50 ON 19 SEP 2006

FILE 'HCAPLUS' ENTERED AT 14:13:50 ON 19 SEP 2006

FILE 'NTIS' ENTERED AT 14:13:50 ON 19 SEP 2006

FILE 'LIFESCI' ENTERED AT 14:13:50 ON 19 SEP 2006


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=> s thioredoxin
L1       27165 THIOREDOXIN

=> s (truncated or modified) and l1
L2        1360 (TRUNCATED OR MODIFIED) AND L1

=> s stain? or dye?
L3     1932122 STAIN? OR DYE?

=> s l2 and l3
L4          61 L2 AND L3

=> s (inclusion (w)bod? ) and l4
L5           2 (INCLUSION (W) BOD? ) AND L4

=> d 1-2 ibib ab

L5    ANSWER 1 OF 2  BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1998-09126  BIOTECHDS
TITLE:            Production of polypeptides as inclusion
                  bodies;
                     recombinant protein preparation by plasmid pTrc99A or
                     plasmid pTrxfus vector-mediated thioredoxin
                     expression in Escherichia coli inclusion
                     body
AUTHOR:           Chatterjee D; Longo M; Flynn E; Oberfelder R
PATENT ASSIGNEE:  Life-Technol.
LOCATION:         Rockville, MD, USA.
PATENT INFO:      WO 9830684 16 Jul 1998
APPLICATION INFO: WO 1998-US492 8 Jan 1998
PRIORITY INFO:    US 1997-34658 8 Jan 1997
DOCUMENT TYPE:    Patent
LANGUAGE:         English
OTHER SOURCE:     WPI: 1998-399134 [34]
AB    A new method for the preparation of a protein in the form of an
```

inclusion body involves: obtaining a host cell (e.g. Escherichia coli) containing a first DNA sequence encoding the protein linked to a second DNA sequence encoding an inclusion partner (thioredoxin or modified thioredoxin), forming a gene fusion construct (plasmid pTrcpr1-monomer or pTrxA-concat); and culturing the cell to favor production of the protein as inclusion bodies. Also new are: vector plasmid pTrc99A and plasmid pTrxfus containing the construct; a host cell containing the vector; making a protein mol.weight ladder composition by obtaining one or more DNA sequences encoding proteins of different mol.weight values, transforming host cells with the DNA, culturing the cells to favor production of each protein, and isolating each protein; and making one or more stained proteins by incubating the proteins with one or more protein-binding dyes under incubation conditions to complex the proteins with the dyes. The methods may be used to prepare a fragment of the gene-32 protein of phage T4, a fragment of KpnI-methylase or a fragment of E. coli Dead-Box protein or thioredoxin. (84pp)

L5 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1998:493673 HCAPLUS
DOCUMENT NUMBER: 129:118770
TITLE: Methods for production of recombinant proteins as inclusion bodies in bacterial host cells
INVENTOR(S): Chatterjee, Deb; Longo, Mary; Flynn, Elizabeth; Oberfelder, Robert
PATENT ASSIGNEE(S): Life Technologies, Inc., USA
SOURCE: PCT Int. Appl., 86 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 9830684	A1	19980716	WO 1998-US492		19980108
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM					
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG					
AU 9860209	A1	19980803	AU 1998-60209		19980108
EP 963435	A1	19991215	EP 1998-903438		19980108
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO					
JP 2001512306	T2	20010821	JP 1998-531177		19980108
US 2002065392	A1	20020530	US 1998-4068		19980108
US 6703484	B2	20040309			
US 2004204563	A1	20041014	US 2003-728766		20031208
PRIORITY APPLN. INFO.:			US 1997-34658P	P	19970108
			US 1998-4068	A1	19980108
			WO 1998-US492	W	19980108

AB The current invention provides methods for producing a polypeptide as inclusion bodies in bacterial host cells. The present methods are carried out by forming a gene construct comprising the genetic sequence encoding a polypeptide operatively linked to that of an inclusion partner protein, such as Escherichia coli thioredoxin or a modified E. coli thioredoxin, such that host cells comprising the gene construct produce the polypeptide as intracellular

inclusion bodies. Addnl. inclusion partner proteins include gene 32 protein of bacteriophage T4, KpnI methylase, and E. coli Dead-Box protein. The methods of the present invention facilitate the rapid isolation and purification of recombinant proteins. In addition, the present methods may be useful for producing polypeptides or proteins which are small and are typically difficult to express, as well as those proteins that are toxic to host cells such as E. coli. The present invention also provides plasmids, vectors and host cells to be used in the present invention for production of polypeptides, and methods of production of polypeptides using these vectors and host cells. The desired protein can be released from inclusion bodies by chemical cleavage (CNBr or hydroxylamine) or enzymic cleavage (factor Xa, thrombin, enterokinase). The invention further provides methods for producing protein mol. weight ladders for use in protein gel electrophoresis, as well as proteins and protein mol. weight ladders produced by these methods.
REFERENCE COUNT: 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:13:50 ON 19 SEP 2006
L1 27165 S THIOREDOXIN
L2 1360 S (TRUNCATED OR MODIFIED) AND L1
L3 1932122 S STAIN? OR DYE?
L4 61 S L2 AND L3
L5 2 S (INCLUSION (W)BOD?) AND L4

=> dup rem l4
PROCESSING COMPLETED FOR L4
L6 27 DUP REM L4 (34 DUPLICATES REMOVED)

=> d 1-27 ibib ab

L6 ANSWER 1 OF 27 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2006-09399 BIOTECHDS
TITLE: Effect of carboxyl-terminal truncation on structure and lipid interaction of human apolipoprotein E4; human C-terminus truncated apolipoprotein-E4 gene transfer and expression in Escherichia coli for recombinant protein production and structure and lipid interaction effect evaluation
AUTHOR: TANAKA M; VEDHACHALAM C; SAKAMOTO T; DHANASEKARAN P; PHILLIPS MC; LUND-KATZ S; SAITO H
CORPORATE SOURCE: Univ Penn; Kobe Pharmaceut Univ
LOCATION: Lund-Katz S, Univ Penn, Childrens Hosp Philadelphia, Sch Med, Lipid Res Grp, Abramson Res Bldg,Suite 1102,3615 Civ Ctr Blvd, Philadelphia, PA 19104 USA
SOURCE: BIOCHEMISTRY; (2006) 45, 13, 4240-4247
ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English
AB AUTHOR ABSTRACT - Apolipoprotein (apo) E4 has been identified as a major risk factor for Alzheimer's disease. Recently, apoE4 was found to undergo proteolytic cleavage in Alzheimer's disease brains, resulting in neurotoxic C-terminal-truncated fragments. In this study, we examined the effect of progressive truncation of the C-terminal domain in apoE4 on its lipid-free structure and lipid binding properties. Circular dichroism measurements demonstrated that deletion of residues 273-299 or 261-299 significantly decreased the number of helical residues, suggesting that the C-terminal residues 261-299 have (alpha-helical

structure. Although the progressive deletions in the C-terminal domain appear to somewhat increase thermal stability, apoE4 (Delta 273-299) and apoE4 (Delta 261-299) showed stability similar to that of the apoE4 22-kDa fragment (residues 1-191) when denatured with guanidine-HCl, indicating that residues 192-272 have a negligible effect on the stability of the C-terminal-truncated apoE4. Comparison of Trp-264 fluorescence in single Trp mutants of full-length and C-terminal-truncated apoE4 (Delta 273-299) indicated that the C-terminal domain structure in the latter is both less organized and cooperative. In addition, comparison of the binding of the C-terminal-truncated mutants to a hydrophobic fluorescent dye and to lipid emulsions revealed that residues 261-272 create a hydrophobic site which is critical for lipid binding. These results suggest that removal of a hydrophobic C-terminal alpha-helical segment (residues 273-299) to create C-terminal-truncated apoE4 forms found in brain leads to less organized C-terminal structure while still retaining a second alpha-helical lipid-binding region (residues 261-272) that is available for interaction with cell membranes and other proteins such as amyloid beta peptide. (8 pages)

L6 ANSWER 2 OF 27 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2006229345 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 16635084
TITLE: Expression of peroxiredoxin and thioredoxin in human lung cancer and paired normal lung.
AUTHOR: Park Joo Hun; Kim Young Sun; Lee Hye Lim; Shim Jin Young; Lee Keu Sung; Oh Yoon Jung; Shin Seung Soo; Choi Young Hwa; Park Kwang Joo; Park Rae Woong; Hwang Sung Chul
CORPORATE SOURCE: Department of Pulmonary and Critical Care Medicine, Ajou University School of Medicine, Suwon, Korea.
SOURCE: Respirology (Carlton, Vic.), (2006 May) Vol. 11, No. 3, pp. 269-75.
Journal code: 9616368. ISSN: 1323-7799.
PUB. COUNTRY: Australia
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 26 Apr 2006
Last Updated on STN: 7 Jun 2006

AB BACKGROUND: Peroxiredoxins (Prxs) have been implicated in regulating many cellular processes including cell proliferation, differentiation and apoptosis. However, the pathophysiological significance of Prx proteins, especially in lung disease, has not been defined. Therefore, the authors investigated the distribution and expression of various Prx isoforms in lung cancer and compared this with normal lung from human and mouse. METHODS: Patients diagnosed with lung cancer who underwent surgery at Ajou Medical Center were enrolled. Expression of Prxs, thioredoxin and thioredoxin reductase was analysed by proteomic techniques. Immunohistochemistry was performed to localize Prx proteins. RESULTS: Immunohistochemical staining showed that the isoforms of Prx I, II, III and V were predominantly expressed in bronchial and alveolar epithelium as well as in alveolar macrophages of the normal mouse lung. The isoforms I, III and thioredoxin were overexpressed in lung cancer tissues compared with normal lungs. There was also an increased amount of oxidized form of Prx I and a putative truncated form of Prx III in lung cancer samples when analysed on two-dimensional electrophoresis. In addition, a 40-kDa intermediate MW protein band and high MW bands of over 20 kDa, recognized by anti-Prx (a-Prx) I antibody, were present in tissue extracts of lung cancer patients on one-dimensional electrophoresis. CONCLUSION: The upregulation of Prx I, Prx III and thioredoxin in lung cancer tissue may represent an attempt by tumour cells to adjust to the microenvironment in a manner that is advantageous to survival and proliferation.

L6 ANSWER 3 OF 27 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2006:245546 HCAPLUS
TITLE: Bioengineered flagella nanotube composites of mesophilic bacteria
AUTHOR(S): Mudalige, Thilak Kumara; Srividya, Narayanan; Tripp, Brian C.; Muralidharan, Subra
CORPORATE SOURCE: Department of Chemistry, Western Michigan University, Kalamazoo, MI, 49008, USA
SOURCE: Abstracts of Papers, 231st ACS National Meeting, Atlanta, GA, United States, March 26-30, 2006 (2006), COLL-006. American Chemical Society: Washington, D. C.
CODEN: 69HYEC
DOCUMENT TYPE: Conference; Meeting Abstract; (computer optical disk)
LANGUAGE: English
AB Protein nanotubes present unique opportunities for the generation of novel composites with inorg. and organic components and the fabrication of nanoscale devices for applications as sensors, separation media, and templates for the generation other nanotube structures. Our research program is focused on engineering flagellin (FliC) proteins and their flagella by site directed mutagenesis in which specific binding groups such as peptide loops have been introduced in the D3 domain. We have engineered FliC proteins of mesophilic bacteria with a thioredoxin loop in the D3 domain which has been further modified with various peptide loops such as histidine and cysteine loops. Various nanoparticles such as Au, Ag, and Cd have been bound to the histidine loops to obtain ordered arrays of nanoparticles on flagella nanotubes. Cu and silica nanotubes have been generated by the reduction of bound Cu2+ ions and the acid hydrolysis of silicate ions. Flagella with cysteine loops self-assemble through the formation of disulfide bonds to yield flagella bundles. These can be dissociated by breaking the disulfide bond by the addition of reducing agents such as TCEP. These bundles can be visualized with a fluorescence microscope by staining with nanoorange. The cysteine loops flagella and their bundles exhibit interesting CD and near IR spectra which indicate their three dimensional structures. Salient results from these studies will be presented.

L6 ANSWER 4 OF 27 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2006011373 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16313183
TITLE: Biophysical and biological studies of end-group-modified derivatives of Pep-1.
AUTHOR: Weller K; Lauber S; Lerch M; Renaud A; Merkle H P; Zerbe O
CORPORATE SOURCE: Drug Formulation and Delivery Group, Department of Chemistry and Applied BioSciences, ETH Zurich, Wolfgang-Pauli-Strasse 10, CH-8093 Zurich, Switzerland.
SOURCE: Biochemistry, (2005 Dec 6) Vol. 44, No. 48, pp. 15799-811. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200601
ENTRY DATE: Entered STN: 10 Jan 2006
Last Updated on STN: 1 Feb 2006
Entered Medline: 31 Jan 2006
AB Pep-1 is a tryptophane-rich cell-penetrating peptide (CPP) that has been previously proposed to bind protein cargoes by hydrophobic assembly and translocate them across cellular membranes. To date, however, the molecular mechanisms responsible for cargo binding and translocation have not been clearly identified. This study was conducted to gain insight into the interaction between Pep-1 with its cargo and the biological membrane to identify the thereby involved structural elements crucial for translocation. We studied three peptides differing in their N- and

C-termini: (i) Pep-1, carrying an acetylated N-terminus and a C-terminal cysteamine elongation, (ii) AcPepWAmide, with an acetylated N-terminus and an amidated C-terminus, and (iii) PepW, with two free termini. Thioredoxin (TRX) and beta-galactosidase were used as protein cargoes. To study CPP-membrane interactions, we performed biophysical as well as biological assays. To mimic biological membranes, we used phospholipid liposomes in a dye leakage assay and surfactant micelles for high-resolution NMR studies. In addition, membrane integrity, cell viability, and translocation efficiency were analyzed in HeLa cells. An alpha-helical structure was found for all peptides in the hydrophobic N-terminal region encompassing residues 4-13, whereas the hydrophilic region remained unstructured in the presence of micelles. Our results show that the investigated peptides interacted with the micelles as well as with the protein cargo via their tryptophan-rich domain. All peptides displayed an orientation parallel to the micelle surface. The C-terminal cysteamine group formed an additional membrane anchor, leading to more efficient translocation properties in cells. No membrane permeabilization was observed, and our data were largely compatible with an endocytic pathway for cellular uptake.

L6 ANSWER 5 OF 27 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2005:993369 SCISEARCH
THE GENUINE ARTICLE: 967TA
TITLE: Protein modifications by 4-hydroxynonenal and 4-hydroxyhexenal in light-exposed rat retina
AUTHOR: Tanito M (Reprint); Elliott M H; Kotake Y; Anderson R E
CORPORATE SOURCE: Univ Oklahoma, Hlth Sci Ctr, Dept Ophthalmol, 608 SL Young Blvd, Oklahoma City, OK 73104 USA (Reprint); Univ Oklahoma, Hlth Sci Ctr, Dept Ophthalmol, Oklahoma City, OK 73104 USA; Univ Oklahoma, Hlth Sci Ctr, Dept Cell Biol, Oklahoma City, OK 73104 USA; Dean A McGee Eye Inst, Oklahoma City, OK USA; Oklahoma Med Res Fdn, Free Rad Biol & Aging Res Program, Oklahoma City, OK 73104 USA
masaki-tanito@ouhsc.edu
COUNTRY OF AUTHOR: USA
SOURCE: INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE, (OCT 2005) Vol. 46, No. 10, pp. 3859-3868.
ISSN: 0146-0404.
PUBLISHER: ASSOC RESEARCH VISION OPHTHALMOLOGY INC, 12300 TWINBROOK PARKWAY, ROCKVILLE, MD 20852-1606 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 37
ENTRY DATE: Entered STN: 13 Oct 2005
Last Updated on STN: 13 Oct 2005

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB PURPOSE. 4-Hydroxynonenal (4-HNE) and 4-hydroxyhexenal (4-HHE) are reactive aldehydes derived from the nonenzymatic oxidation of n-6 and n-3 polyunsaturated fatty acids, respectively. Increasing evidence suggests that protein modifications by reactive aldehydes are involved in various diseases. The present study was undertaken to test whether protein modifications by 4-HNE and 4-HHE increase in retinal tissues after exposure of rats to damaging levels of light.

METHODS. Albino rats were exposed to 1 or 5 klux white fluorescent light for 3 hours and, at various times thereafter, the levels and localizations of aldehyde-modified proteins in retinas were assessed by densitometric analysis of semiquantitative Western dot blots and by immunohistochemistry, using 4-HNE- and 4-HHE-specific antibodies. In some rats, the protective antioxidant phenyl-N-tert-butylnitrone (PBN) was injected (50 mg/kg) before exposure to light. To assess retinal damage, outer nuclear layer (ONL) thickness was measured on hematoxylin-eosin (H&E)-stained sections, and apoptosis was semi-quantitatively analyzed by TUNEL staining.

RESULTS. By dot blot analysis, 4-HNE- and 4-HHE-modified proteins were significantly increased in retina (both by 1.7-fold) and RPE fraction (1.5- and 1.8-fold, respectively) after 5-klux exposure. In retina, increases in 4-HNE- and 4-HHE-modified proteins were more prominent at 3 hours than at 24 hours or 48 hours after exposure to light. In rod outer segments, only 4-HHE-modified proteins increased significantly (1.4-fold). Retinal thinning, TUNEL staining in ONL, 4-HNE-, and 4-HHE protein modifications were all found in the same retinal regions. PBN treatment inhibited the light-induced increase of 4-HNE and 4-HHE modified proteins in retina and RPE fractions.

CONCLUSIONS. Exposure to intense light increases 4-HNE and 4-HHE protein modifications in the retina, suggesting that free radical initiated, nonenzymatic reactions are involved in this process. These modifications may be early events that precede photoreceptor cell apoptosis.

L6 ANSWER 6 OF 27 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2005266007 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15907482
TITLE: NMR structure and regulated expression in APL cell of human SH3BGRL3.
AUTHOR: Xu Chao; Zheng Peizheng; Shen Shuhong; Xu Yingqi; Wei Ling; Gao Hengjun; Wang Shengnian; Zhu Chongri; Tang Yajun; Wu Jihui; Zhang Qinghua; Shi Yunyu
CORPORATE SOURCE: Hefei National Laboratory for Physical Sciences at Microscale, School of Life Sciences, University of Science and Technology of China.
SOURCE: FEBS letters, (2005 May 23) Vol. 579, No. 13, pp. 2788-94. Electronic Publication: 2005-04-20. Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200506
ENTRY DATE: Entered STN: 24 May 2005
Last Updated on STN: 1 Jul 2005
Entered Medline: 30 Jun 2005

AB SH3 domain binding glutamic acid-rich protein like 3 (SH3BGRL3) is the new member of thioredoxin (TRX) super family, whose posttranslational modified form was identified as tumor necrosis factor alpha (TNF-alpha) inhibitory protein, TIP-B1. In this paper, we determined its solution structure by multi-dimensional nuclear magnetic resonance spectroscopy. The overall structure of human SH3BGRL3 conformed to a TRX-like fold. To understand its function in vivo, the upregulated expression in acute promyelocytic leukemia cell line NB4 at both mRNA and protein level was elucidated. Immunofluorescence and immunohistochemistry staining with monoclonal antibody against SH3BGRL3 demonstrated that it was a cytoplasmic protein in both NB4 cell and human tissues. These results, as a whole, indicate that SH3BGRL3 may function as a regulator in all-trans retinoic acid-induced pathway.

L6 ANSWER 7 OF 27 EMBASE
ACCESSION NUMBER: 2005495214 EMBASE
TITLE: Expression of peroxiredoxin and thioredoxin in human lung cancer and paired normal lung.
AUTHOR: Kim Y.S.; Park J.H.; Lee H.L.; Shim J.Y.; Choi Y.I.; Oh Y.J.; Shin S.S.; Choi Y.H.; Park K.J.; Park R.W.; Hwang S.C.
CORPORATE SOURCE: S.C. Hwang, Dept. of Pulomonary and Critical Care Medicine, Ajou University School of Medicine, Suwon 442-749, Korea, Republic of. schwang@ajou.ac.kr

SOURCE: Tuberculosis and Respiratory Diseases, (2005) Vol. 59, No. 2, pp. 142-150. .
Refs: 29
ISSN: 1738-3536 CODEN: KHCHAM
COUNTRY: Korea, Republic of
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
015 Chest Diseases, Thoracic Surgery and Tuberculosis
016 Cancer
029 Clinical Biochemistry
LANGUAGE: Korean
SUMMARY LANGUAGE: English; Korean
ENTRY DATE: Entered STN: 1 Dec 2005
Last Updated on STN: 1 Dec 2005

AB Background: Continuous growth stimulation by various factors, as well as chronic oxidative stress, may co-exist in many solid tumors, such as lung cancer. A new family of antioxidant proteins, the peroxiredoxins (Prxs), have been implicated in the regulation of many cellular processes, including cell proliferation, differentiation and apoptosis. However, a real pathophysiological significance of Prx proteins, especially in lung disease, has not been sufficiently defined. Therefore, this study was conducted to investigate the distribution and expression of various Prx isoforms in lung cancer and other pulmonary conditions. Method: Patients diagnosed with lung cancer, and who underwent surgery at the Ajou Medical Center, were enrolled. The expressions of Prxs, Thioredoxin (Trx) and Thioredoxin reductase (TR) were analyzed using proteomic techniques and the subcellular localization of Prx proteins was studied using immunohistochemistry on normal mouse lung tissue. Result: Immunohistochemical staining has shown the isoforms of Prx I, II, III and V are predominantly expressed in bronchial and alveolar lining epithelia, as well as in the alveolar macrophages of the normal mouse lung. The isoforms of Prx I and III, and thioredoxin were also found to be over expressed in the lung cancer tissues compared to their paired normal lung controls. There was also an increased amount of the oxidized form of Prx I, as well as a putative truncated form of Prx III, in the lung cancer samples when analyzed using 2 dimensional electrophoresis. In addition, a 43 kDa intermediate molecular weight protein band, and other high molecular weight bands of over 20 kDa, recognized by the anti Prx I antibody, were present in the tissue extracts of lung cancer patients on 1 Dimensional electrophoresis, which require further investigation. Conclusion: The over expressions of Prx I and III, and Trx in human lung cancer tissue, as well as their possible chaperoning function, may represent an attempt by tumor cells to adjust to their microenvironment in a manner advantageous to their survival and proliferation, while maintaining their malignant potential.

L6 ANSWER 8 OF 27 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2006:195198 HCAPLUS
TITLE: Avoidance of oxidative-stress perturbation in yeast bioprocesses by proteomic and genomic biostrategies?
AUTHOR(S): Wiseman, A.
CORPORATE SOURCE: Molecular Toxicology Group, School of Biomedical & Life Sciences, University of Surrey, Guildford, Surrey, UK
SOURCE: Letters in Applied Microbiology (2005), 40(1), 37-43
CODEN: LAMIE7; ISSN: 0266-8254
PUBLISHER: Blackwell Publishing Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Aims: Bioprocess oxidative stress caused by many reactive oxygen species (ROS) can lead to largely irreversible perturbation of yeast bioprocesses. These include the production of proteins derived from recombinant DNA yeast technol. (aerobically grown Saccharomyces cerevisiae). These proteins include rennin, amyloglucosidases (glucamylases), interferons,

interleukins, insulin, monoclonal antibodies, tissue plasminogen activators (t-PA), sexually transmitted disease antigens, and measles, mumps and rubella antigens, growth hormones, somatotropin, blood clotting factors VIII and XIII. In addition, there may be a demand for severe acute respiratory syndrome-coronavirus antigens, hepatitis A, B and C viral-selected antigens, HIV retroviral antigens, influenza antigens, trypanosomal antigens, and foot and mouth disease antigens. Prevention of oxidative stress has been achieved by application of antioxidant redox metalloenzymes such as superoxide dismutases (containing Cu/Zn cytosolic, Mn mitochondrial and Fe bacterial) glutathione peroxidases (and other Se-containing proteins and enzymes such as the thioredoxins), catalases (Fe-containing), cytochrome c peroxidases (Fe-containing), ceruloplasmins (Cu-containing), metallothionines (these cysteine thiol-rich proteins bind ions of cadmium and mercury) and tyrosinases (Cu-containing). Methods and Results: ROS are generated inadvertently by single metal valency couples such as FeII/FeIII and by FeIII/FeV present in 2700 (including 57 human) isoforms in cytochromes P 450 mixed-function oxidases (EC 1.14.14.1; O2 : mono-oxygenase NADPH/NADH requiring). In addition, mixed-metal couples such as valency unmatched forms in CuI/FeII and FeIII/MnIV can recycle electrons. Moreover, proteins/protein chaperone couples can recycle electrons, often where futile-recycling systems have been instigated. Furthermore, oxidized membrane phospholipids (R) can form ROOH (lipid hydroperoxides) and ROH (lipid alkoxides) that can generate ROS through Fenton chemical (iron-catalyzed) chain reactions. Utilization of chain-breaking antioxidants such as vitamin E (α-tocopherol) in the lipid phase and vitamin C (ascorbate) in the aqueous phase can terminate these ROS-producing reactions. Conclusions: The main significance of the study is that proteomic strategies of relief from bioprocess perturbation by ROS of yeast fermns. (used to manufacture proteins required in the food and therapeutic bioindustries) may become possible through addition of selected proteins (including metalloenzymes). The main impact of the study is that the utilization of genetically modified (GM) yeast produced by recombinant DNA technol. genomic strategies could circumvent the bioprocessing problems that otherwise result from the bioprocess perturbations: this is as a result of oxidative stress caused by ROS, which is avoidable by deployment of appropriate antioxidants such as vitamins E, C and D (and antioxidant proteins and enzymes often of microbial origin via recombinant DNA technol.).

REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 9 OF 27 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2004-14777 BIOTECHDS

TITLE: Novel Escherichia coli host cell producing recombinant antibody, genetically modified in order to change physical property of proteins of wild-type Escherichia coli, that co-purify with recombinant antibody; recombinant antibody production via plasmid expression in host cell

AUTHOR: HUMPHREYS D P; CHAPMAN A P; ROBINSON M K; SPITALI M

PATENT ASSIGNEE: CELLTECH R and D LTD

PATENT INFO: WO 2004035792 29 Apr 2004

APPLICATION INFO: WO 2003-GB4474 15 Oct 2003

PRIORITY INFO: GB 2002-24082 16 Oct 2002; GB 2002-24082 16 Oct 2002

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2004-389520 [36]

AB DERWENT ABSTRACT:

NOVELTY - An Escherichia coli host cell (I) expressing a recombinant antibody, is genetically modified in order to change at least one physical property of one or more proteins of wild-type E. coli, that co-purify with the recombinant antibody, is new.

BIOTECHNOLOGY - Preferred Host cell: In (I), the physical characteristic of the E. coli protein that is altered is the isoelectric

point, hydrophobicity or size, preferably isoelectric point. The altered host protein is phosphate binding protein (PhoS/PstS), dipeptide protein (DppA), maltose binding protein (MBP) or thioredoxin 1, preferably PhoS/PstS. The isoelectric point of the host protein is altered by the addition of a poly-aspartic acid tag to the C-terminus. The isoelectric point of the PhoS/PstS has been reduced by substituting one or more lysines at amino acid 110, 265, 266 or 318 with glutamine or aspartic acid and further by the addition of a poly-aspartic acid tag to the C-terminus, preferably by substituting the lysines at residues 265 and 266 with glutamine and by the addition of a poly-aspartic acid tag to the C-terminus or by substituting the lysines at residues 110, 265 and 266 with glutamine and by the addition of a poly-aspartic acid tag to the C-terminus. The recombinant antibody is a Fab or Fab' fragment.

USE - (I) is useful for producing a recombinant antibody, which involves fermenting (I) (claimed).

ADVANTAGE - (I) is naturally acquired organism or mutated organism capable of efficiently producing recombinant antibodies. (I) improves the purification process of antibody during fermentation. The recombinant antibody is produced by (I) at lower cost and within shorter period of time.

EXAMPLE - Strain Escherichia coli DPH3 was transformed with a plasmid expressing the desired recombinant antibody (Fab') and altered phosphate binding protein (PhoS). A standard fermentation was performed. Samples taken throughout the fermentation were assayed by enzyme linked immunosorbent assay (ELISA) after Tris/ethylenediaminetetraacetic acid (EDTA) extraction. After fermentation, centrifugation was carried out and pellets representing 50 ml of harvest culture were extracted overnight at 30 degrees C in Tris/EDTA then prepared for cation exchange purification. The pH was increased from 4.5-5.0 so that PhoS of strain DPH3 would not bind to the cation exchange column but the Fab' fragment would. The conductivity was 3.0 mS/cm. The samples was applied to a 5 ml SP sepharose column and load, the obtained fractions were analyzed by Coomassie stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Samples were concentrated by using 10 kDa cut-off columns to enable visualization with Coomassie stained gels. The SDS-PAGE gel showed that under the pH and conductivity conditions the mutant PhoS from DPH3 does not bind to the SP sepharose column, while wild-type PhoS (wt PhoS) from E. coli W3110 strain (wild-type) does bind. The means that in DPH3 the mutant PhoS and Fab' appeared in different fractions, while in W3110 both proteins appeared in same fraction. To confirm that any remaining PhoS would also be removed by anion exchange, the fractions from both DPH3 and W3110 experiments were concentrated, desalted and buffer exchanged to 20 mM Tris chloride and run on anion exchange. The Coomassie stained SDS-PAGE gel showed that for DPH3 PhoS binds to the anion exchange column hence separating it from the Fab'. While, the wt PhoS from W3110 does not bind to the column and flows through and contaminates the Fab' solution. Thus on altering the expression of PhoS protein, enabled efficient purification of recombinant antibody (Fab'). (60 pages)

L6 ANSWER 10 OF 27 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-13947 BIOTECHDS
TITLE: Novel polypeptide antagonist to peripheral-type benzodiazepine receptor, useful for treating disease or condition such as breast cancer, multiple sclerosis, cirrhosis, ischemia and Alzheimer's disease; antagonist against receptor for use in disease therapy and gene therapy
AUTHOR: PAPADOPOULOS V; GAZOULI M
PATENT ASSIGNEE: UNIV GEORGETOWN
PATENT INFO: WO 2004031722 15 Apr 2004
APPLICATION INFO: WO 2003-US29822 23 Sep 2003
PRIORITY INFO: US 2002-414635 1 Oct 2002; US 2002-414635 1 Oct 2002
DOCUMENT TYPE: Patent

LANGUAGE: English
OTHER SOURCE: WPI: 2004-348000 [32]
AB DERWENT ABSTRACT:
NOVELTY - A polypeptide antagonist (I) to the peripheral-type benzodiazepine receptor (PBR), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) identifying (M1) an antagonist to the PBR, involving: (a) obtaining a library comprising random polypeptides; (b) screening the library to identify polypeptides that bind to PBR; and (c) testing the identified polypeptides for the ability to prevent binding of a recognized PBR ligand, where the identified polypeptide that prevents binding of a PBR ligand is an antagonist of PBR; (2) an antagonist (II) of the PBR identified by (M1); (3) screening (M2) for compounds which are antagonists to the PBR receptor, involving: (a) attaching PBR or its biologically-active polypeptide fragment to a substrate; (b) exposing the PBR or its biologically-active polypeptide fragment to one or more compounds; (c) determining whether the compound is bound to the PBR or its biologically-active polypeptide fragment; and (d) if the compound is bound, further determining whether the compound modulates the interaction between the PBR ligand and the PBR; (4) a compound identified by (M2); (5) a composition (III) for treating a PBR-related disease or condition, comprising a compound which acts as an antagonist to the PBR; (6) preparing (III), involves combining an antagonist to the PBR and a carrier; (7) a kit (IV) comprising an antagonist to the PBR; (8) a compound identified using (II); (9) an isolated polypeptide (V) comprising a motif (MF) Ser-Thr-XXXX-Pro, where the polypeptide modulates PBR and X represents any amino acid; (10) an antibody (VI) or its biologically-active fragment which recognizes and binds to PBR antagonist amino acid sequence which contains MF; (11) a nucleic acid (VII) encoding (I); (12) a vector comprising (VII); and (13) an antagonist of the PBR which is not a polypeptide, where the agonist is a peptide mimetic designed to correspond to a polypeptide antagonist of PBR.

WIDER DISCLOSURE - Detecting the ability of a test sample to affect the binding interaction of a first polypeptide and a second polypeptide of a polypeptide binding pair, where the first polypeptide is a PBR antagonist polypeptide and the second polypeptide is PBR is also disclosed.

BIOTECHNOLOGY - Preferred Antagonist: (I) blocks agonists of the PBR by competitive binding at the ligand binding site of the PBR, where the polypeptide contains MF, and X represents any amino acid. The polypeptide comprises an amino acid sequence (S1) Ser-Thr-Pro-His-Ser-Thr-Pro, which is linked to the TAT sequence derived from HIV. Preferred Kit: In (IV), the antagonists comprises a polypeptide which contains MF. The polypeptide comprises (S1). Preferred Antibody: In (VI), the amino acid sequence comprises (S1). Preferred Polypeptide: (V) comprises (S1), and further comprises TAT sequence of HIV. Preferred Nucleic Acid: In (VII), the polypeptide antagonist is an aptamer, comprising a nucleic acid encoding a scaffold protein in-frame with the polypeptide aptamer. The scaffold protein is thioredoxin A.

ACTIVITY - Cytostatic; Neuroprotective; Tranquilizer; Antidepressant; Nootropic; Hepatotropic; Vasotropic; Antiparkinsonian; Vulnerary.

MECHANISM OF ACTION - Antagonist of PBR; Blocks agonist of PBR by competitive binding at the ligand binding site of the PBR; Regulator of PBR activity (claimed). In vitro analysis of PBR antagonist in inhibiting steroid biosynthesis, was carried out as follows: MA-10 Leydig cells were incubated in the presence of increasing concentrations of TAT-Ser-Thr-Pro-His-Ser-Thr-Pro for 30 minutes. Cells were then exposed to 1 microM of Ro5-4864 (4'-chlorodiazepam). After 4 hours, steroid secreted into the media, was measured by radioimmunoassay. The result indicated inhibition of steroid formation by the sequence with an ED50 of 5 microM. Thus, the sequence was found to act as a competitive PBR antagonist.

USE - (I) is useful for treating a PBR-related disease or condition

in a subject such as canine, feline, ovine, equine, porcine, caprine, camelid, avian, bovine, amphibian, fish, murine or primate organism such as human, which involves administering (I) to the subject, where the PBR-related disease or condition is chosen from breast cancer, colon cancer, prostate cancer, multiple sclerosis, alcohol withdrawal, affective disorders, anxiety disorders, seizures, brain tumors, Alzheimer's disease, stress, acute myeloid leukemia, liver cancer, cirrhosis, traumatic brain injury, ischemia, reactions associated with chemical toxins, and their combinations. (I) is useful for regulating a PBR activity in vivo, which involves administering (I), where (I) modulates PBR by binding with the ligand binding site of PBR. (II) is useful for identifying one or more agents that modulate PBR ligand binding, which involves binding a PBR or a ligand binding domain of PBR to a solid support, adding (II) to the PBR or its fragment which binds to PBR in the presence of the agent, measuring the amount of the ligand binding domain of PBR bound to (II) on the support in the presence of the agent, and identifying an agent that reduces the amount of the ligand binding domain of PBR binding to (II) on the support, where the solid substrate is a microarray. (III) is useful for treating the above-mentioned diseases (claimed). (I) is useful for treating disease or condition such as Parkinson's disease and ovarian cancer.

ADMINISTRATION - (I) is administered by inhalation, subcutaneous, intramuscular, intravenous injection, or by oral route. No specific dosage details are given.

EXAMPLE - Purified human chorionic gonadotrophin (hCG) was obtained. MA-10 cells were grown in modified Waymouth's MB752/1 medium containing 15% horse serum. Mitochondria were isolated. A polypeptide library comprising 1.28 x 10 to the power of 9 possible 7-mer sequences, were obtained. As protein target, MA-10 isolated mitochondria were used. In brief, a microtiter well was coated overnight with 150 microl of 100 microg/ml MA-10 mitochondria in 1X phosphate buffered saline (PBS), using 0.1 M sodium bicarbonate (pH of 8.6) and blocked with a blocking solution containing 0.1 M sodium bicarbonate (pH of 8.6), 5 mg/ml bovine serum albumin (BSA) and 0.02% sodium nitrite. Peripheral-type benzodiazepine receptor (PBR) function on coated mitochondria was examined using radioligand binding assays. About 2 x 10 transducing units of phage from the library were combined and incubated for 1 hour with the coated well at room temperature in 100 microl TBST buffer (50 mM Tris-hydrochloric acid (pH of 7.6), 150 mM sodium chloride and 0.1% (v/v) Tween-20). Wells were washed 10 times with TBST and eluted with 0.1 M of 1-(2-chlorophenyl)-N-methyl-N- (1-methyl-propyl)-3-isoquinoline carboxamide (PK 11195). DNA from individual phage clones was sequenced after three rounds of selection. Single-stranded DNA from individual phage clones was purified. Nucleotide sequences were analyzed on a DNA sequencer using the sequencing primer having sequence of 5'-HO-CCCTCATAGTTAGCGTAACG-3'. Radioligand binding assays were performed. MA-10 mitochondria were suspended in 1X PBS at a final concentration of 10 microg protein/100 microl. Specific radioligand binding (2.5 nM) to MA-10 mitochondria was measured in the presence or absence of increasing concentrations (10 to the power of -6 M to 10 to the power of -3 M) of competing PBR peptides. In all cases after 120 minutes of incubation at 4 degrees C, the incubation reaction was stopped by filtration through filters. Bound radioactivity was determined by liquid scintillation spectrometry. Twenty-mer TAT-Ser-Thr-Pro-His-Ser-Thr-Pro and TAT-Ser-Thr-His-Glu-Glu-Thr-Pro peptides were synthesized that contained an NH2-terminal 11-mer TAT protein transduction domain, followed by two glycine residues. Transduction experiments were performed. To determine the efficiency of TAT polypeptide incorporation into the cells, MA-10 cells were cultured overnight on 8-chambered Super Cell Culture Slides at a concentration of approximately 25000 cells/chamber. Media were replaced 24 hours later with fresh media and cells were treated with various concentrations of Oregon Green 488-labeled peptides for various time periods. After the incubation period, cells were washed with PBS and examined by fluorescent microscopy. For steroid synthesis experiments,

MA-10 cells were plated into 96-well plate at the density of 2.5 x 10 to the power of 4 cells/well. About 24 hours later, media was replaced with fresh media and cells were treated with the indicated concentrations of peptides for 30 minutes. Cells were then stimulated with 50 ng/ml hCG or 20 microM of the hydrosoluble analog of cholesterol, 22 R-hydrocholesterol in serum-free media for 2 hours. At the end of the incubation, culture media were collected and tested for progesterone production by radioimmunoassay using anti-progesterone antisera. Progesterone production was normalized against the amount of protein in each well. Proteins were quantified using dye-binding assay with bovine serum albumin as standard. In biopanning experiments, PBR interacting phage peptides were eluted using PK 11195 or Ro5-4864 (4'-chlorodiazepam). After three rounds of panning, 20 individual polypeptide clones from each eluate were selected, amplified and sequenced. The polypeptide sequences were found to comprise a motif Ser-Thr-XXXX-Pro. (31 pages)

L6 ANSWER 11 OF 27 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-11990 BIOTECHDS
TITLE: Novel polypeptide sharing one or more homologue amino acid domains with Legless protein being functional homologue of Legless, useful for diagnosing disorders of cell fate; vector-mediated gene transfer and expression in host cell for recombinant protein production, drug screening and gene therapy
AUTHOR: BASLER K; BRUNNER E; FROESCH B; KRAMPS T; PETER O
PATENT ASSIGNEE: UNIV ZURICH
PATENT INFO: US 2004038901 26 Feb 2004
APPLICATION INFO: US 2003-664859 22 Sep 2003
PRIORITY INFO: US 2003-664859 22 Sep 2003; US 2000-221502 28 Jul 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-203288 [19]
AB DERWENT ABSTRACT:
NOVELTY - A polypeptide (I) sharing one or more homologue amino acid domains with a Legless protein being functional homologue of Legless, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a nucleotide sequence (II) coding for a protein present in invertebrate and/or vertebrate organisms, the nucleotide sequence coding for a protein comprising a positive function in a regulatory pathway; (2) a vector (III) comprising (II); (3) a host cell (IV) containing (III) chosen from mammalian, bacterial, yeast, plant and insect cells; (4) use of (I) for the isolation of Lgs-binding proteins by carrying out an assay chosen from in vitro binding assay with such a peptide, or a co-immunoprecipitation from vertebrate or invertebrate cell lysates or a mammalian or yeast two hybrid assay, preferably co-immunoprecipitation assay; (5) producing (I); (6) an antibody (V) which specifically binds to (I), chosen from polyclonal antibodies, monoclonal antibodies, humanized antibodies and single chain antibodies; (7) a chimeric molecule (VI) comprising (I) or its fragment fused to heterologous amino acid sequence; (8) a peptide (VII) comprising a stretch of amino acids comprising one or more sequence homology domain, which is common between the Drosophila Legless and human Legless proteins; (9) a compound (VIII) interfering with the binding to the domains of (VII), for inhibiting the interaction between partner proteins to these domains by exposing the domains to the compounds; (10) use of (VIII) in a pharmaceutical composition delivering the peptide or its relative nucleic acid sequence in an appropriate vector into a cancerous cell; (11) a synthetic molecule, simulating the function of Legless proteins in the Wnt pathway; (12) an antagonist of (I) chosen from comprising small bioorganic molecules, synthetic polymers, or small polypeptides; (13) an agonist of (I) chosen from comprising small polypeptides, and small bioorganic molecules; (14) screening (M1) for agonists and/or antagonists of (I) for functional

activity; (15) an antisense oligonucleotide sequence (IX), derived from (II); (16) use of the antisense oligonucleotide in the therapy of diseases caused by an over-activation of the Wg pathway; (17) a double-stranded RNA sequence (X) derived from (II) comprising RNA interfering activities; (18) use of (X) for reducing lgs gene expression in an invertebrate or vertebrate organism or invertebrate or vertebrate cell line; and (19) a pharmaceutical composition (XI) comprising an oligonucleotide derived from (II), and carrier, where the oligonucleotide and carrier being capable of passing through a cell membrane; (20) a pharmaceutical composition comprising derived from (I) and further comprises carrier, the pharmaceutical composition being an oligonucleotide and the pharmaceutical carrier are capable of passing through a cell membrane.

BIOTECHNOLOGY - Preparation: Producing (I), involves culturing (IV) under conditions suitable for expression of (I), and recovering (I) or its fragments from the cell culture (claimed). Preferred Polypeptide: (I) is derived from any one of the sequence of (II). (I) comprises the function of legless proteins. The functional homologue is hLgs/Bcl-9 protein or its fragment, comprising the function of legless protein in Wnt-pathway. Preferred Nucleic Acid: In (II), the pathway is the Wnt/Wg-pathway. The function of the encoded protein comprises the function of legless (lgs) gene products. The nucleotide sequence is coding for Drosophila Legless (Lgs) protein. The nucleotide sequence comprises the a fully defined sequence (S1) as given in the specification. The nucleotides sequence is coding for human Legless (hLgs) proteins. The nucleotide sequence include a fully defined sequence (S2) as given in the specification. The nucleotide sequence comprising 50% or more homology to the nucleotide sequence of stretches of the nucleotide sequence of (S1) or (S2), its complements or fragments. Preferred Vector: (III) operably linked to control sequences recognized by a host cell transformed with the vector. Preferred Chimeric Molecule: In (VI), the heterologous amino acid sequence is chosen from an epitope tag sequence, glutathione-S-transferase group, thioredoxin group, and an antibody group. Preferred Peptide: In (VII), the common domains from human Legless are derived from hLgs-1 or hLgs/Bc19. Preferred Compound: In (VIII), the partner proteins are Doll and beta-catenin. The compounds are chosen from small peptides, synthetic polymers, and natural or synthetic chemical compounds. The compound is a small peptide comprising the sequence homology domain 1 or 2 as given in the specification. Preferred Method: (M1) is a HTRF based protein-protein-interaction assay. Preferred Antisense Oligonucleotide: In (IX), the oligonucleotide sequence hybridizes to RNA and/or genomic DNA encoding a vertebrate Lgs. The oligonucleotide sequence prevents translation of the RNA or transcription of the DNA. The oligonucleotide sequence comprises chemically modified nucleotides or nucleotide analogs. Preferred RNA Sequence: In (X), the double-stranded RNA sequence is able to induce degradation of lgs single stranded RNA. Preferred Composition: In (XI), the oligonucleotide is capable of reducing the expression of a mammalian Lgs protein. The oligonucleotide is coupled to a group that inactivates mRNA. The group inactivating mRNA is a ribozyme (ribozyme is an enzyme). The carrier comprises a structure binding to a receptor on a cell surface, the structure being taken up by the cell after binding to the receptor. The oligonucleotide is (X). The double stranded RNA molecule comprises 18-100 nucleotides, preferably 20-500 nucleotides, more preferably 20-50 nucleotides and most preferably 20-22 nucleotide.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - Administering legless protein; Immunotherapy; Antisense therapy. No supporting data is given.

USE - (I), (II), or its function homologue are useful for treating (M2) disorders of cell fate, which involves administering therapeutic compound chosen from invertebrate and vertebrate Lgs protein homologues or its fragments, antibodies, antibody fragments, Lgs antisense DNA, lgs antisense RNA, lgs double-stranded RNA, small peptides, chemical and

natural compounds being capable of interfering with Lgs function, synthesis and degradation. The disorders of cell fate being differentiation or proliferation. In (M2), the compound is administered to treat a cancerous condition. The therapeutic compound is administered to prevent progression from a pre-neoplastic or non-malignant condition to a neoplastic or malignant state. The therapeutic compound is administered to treat a cancerous condition characterized by over-stimulation of the Wnt pathway. The cancerous condition is colon, breast, head and neck, brain, thyroid, medulloblastoma or skin cancer. The therapeutic compound is administered to a blood disease. The therapeutic compound is administered to promote tissue regeneration and repair. (I), (II) or anti-Lgs antibodies useful for diagnosing disorders of cell fate (claimed).

EXAMPLE - Legless (Lgs) genes was found by positional cloning. DNA encoding full-length or a truncated Lgs form was fused down-stream of an epitope tag or glutathione-S-transferase (GST) cDNA and a thrombin cleavage site contained within an inducible bacterial expression vector. Such epitope tags include poly-his, S-protein, thioredoxin, and immunoglobin tags. A variety of plasmids was employed, including commercially available plasmid such as pGEX-4T. Briefly, a bacterial expression plasmid containing the Lgs sequence, for instance fused to a GST-sequence was transformed by conventional methods into protease deficient Escherichia coli such as BL21. A bacterial colony containing the plasmid was then expanded overnight in selection medium to reach saturation. The next morning, the culture was diluted 1:100 and bacterial were allowed to grow to an optical density (OD600) of 0.6. Protein production was an initiated GST-Lgs fusion protein was expressed. A variety of inducers was employed depending on the expression vector used, including IPTG. Expressed GST tagged Lgs was purified, for instance, using affinity beads or affinity chromatography, such as glutathione beads. Extracts were prepared by lysing the Lgs-expressing bacteria in sonication buffer, followed by short sonication on ice and centrifugation. Cleared supernatants were then incubated under gentle rotation for example with glutathione beads for 2 hours at 4 degreesC. Next beads were washed several time in washing buffer, and finally stored in storage buffer. Alternatively, a His-tagged or IgG tagged Lgs were purified using Ni2+ - chelate affinity chromatography or protein A or protein G column chromatography, respectively. The quality of the preparations was checked by SDS-gel electrophoresis and silver staining or Western blot. The purified proteins were used generate anti-Lgs.(52 pages)

L6 ANSWER 12 OF 27 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:100505 HCAPLUS
DOCUMENT NUMBER: 140:160127
TITLE: Method for identifying apoptosis-modified proteins
INVENTOR(S): Rudel, Thomas; Thiede, Bernd; Machuy, Nikolaus
PATENT ASSIGNEE(S): Germany
SOURCE: U.S. Pat. Appl. Publ., 42 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004022779	A1	20040205	US 2002-297619	20021216
EP 1164374	A1	20011219	EP 2000-112813	20000616
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
WO 2001096873	A2	20011220	WO 2001-EP6780	20010615
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,				

CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT,
RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US,
UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,
BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: EP 2000-112813 A 20000616
EP 2000-125013 A 20001116
WO 2001-EP6780 W 20010615

AB The present invention relates to a method for characterizing or identifying apoptosis-modified proteins which are expressed by cells, preferably human cells. Further, novel apoptosis-modified proteins are provided which are suitable as targets for diagnosis, prevention or treatment of diseases, particularly hyperproliferative or degenerative diseases. Apoptosis was induced in Jurkat T-cells by treatment with an anti-Fas antibody for six hours. Two-dimensional electrophoresis (2-DE) gels were produced after lysis of the cells and separation of the proteins. Approx. 2000 spots were resolved and detected by silver staining. Ten 2-DE gels of apoptotic cells were compared with ten 2-DE gels of Jurkat T-cells. Protein patterns of apoptosis-induced cells and control cells were found to be highly reproducible. In Fas-induced Jurkat T-cells 24 addnl. spots and in untreated Jurkat T-cells 21 addnl. spots were observed Coomassie stained 2-DE gels were used for the identification by mass spectrometry. Results indicate that predominantly cleavage events have occurred within the identified proteins during the apoptotic process. The most striking feature of the identified apoptosis-modified proteins of the total cell lysate is that eight of the proteins contain the RNP-binding motif and seven of the eight proteins, with the exception of nucleolin, are involved in the splicing process.

L6 ANSWER 13 OF 27 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2004290565 EMBASE
TITLE: Identification of cellular proteins modified in response to African swine fever virus infection by proteomics.
AUTHOR: Alfonso P.; Rivera J.; Hernaez B.; Alonso C.; Escribano J.M.
CORPORATE SOURCE: Dr. J.M. Escribano, Departamento de Biotecnologia (INIA), Carretera de la Coruna, km 7,5, E-28040 Madrid, Spain. escriban@inia.es
SOURCE: Proteomics, (2004) Vol. 4, No. 7, pp. 2037-2046. .
Refs: 67
ISSN: 1615-9853 CODEN: PROTC7
COUNTRY: Germany
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
005 General Pathology and Pathological Anatomy
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 29 Jul 2004
Last Updated on STN: 29 Jul 2004

AB Changes in gene expression are produced in cells as a consequence of virus infections. In the present work, we used proteomic technology to globally examine African swine fever virus (ASFV)-infected Vero cells searching for infection-associated proteins in order to determine target proteins for pathogenesis studies. We studied the alterations in cellular protein profile after ASFV infection by two-dimensional electrophoresis, identifying the modified cellular proteins by matrix-assisted laser desorption/ionization peptide mass fingerprinting. A total of twelve different over-expressed cellular proteins were unambiguously

identified. The most significant changes were in redox-related proteins, nucleoside diphosphate kinases, heat shock proteins, members of the Ran-Gppnhp-Ranbd1 complex and apolipoproteins. These cellular protein modifications could represent distinct roles during infection related to apoptosis and transcriptional modulation mechanisms. The present study constitutes the first attempt to understand the dynamics of ASFV-host cell interactions by proteomics.

L6 ANSWER 14 OF 27 MEDLINE on STN
ACCESSION NUMBER: 2003328574 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12857815
TITLE: Potato plants lacking the CDSP32 plastidic thioredoxin exhibit overoxidation of the BAS1 2-cysteine peroxiredoxin and increased lipid Peroxidation in thylakoids under photooxidative stress.
AUTHOR: Broin Melanie; Rey Pascal
CORPORATE SOURCE: Commissariat a l'Energie Atomique (CEA)/Cadarache, Direction des Sciences du Vivant, Departement d'Ecophysiologie Vegetale et de Microbiologie, Laboratoire d'Ecophysiologie de la Photosynthese, France.
SOURCE: Plant physiology, (2003 Jul) Vol. 132, No. 3, pp. 1335-43. Journal code: 0401224. ISSN: 0032-0889.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200311
ENTRY DATE: Entered STN: 15 Jul 2003
Last Updated on STN: 8 Nov 2003
Entered Medline: 7 Nov 2003

AB The CDSP32 protein (chloroplastic drought-induced stress protein of 32 kD) is a thioredoxin participating in the defense against oxidative damage. We recently have identified in vitro the BAS1 2-Cys peroxiredoxin, a peroxide-detoxifying enzyme, as a target for CDSP32. Here, we report the characterization under stress conditions of transgenic potato (Solanum tuberosum) plants lacking CDSP32 with regard to the BAS1 redox state and the level of lipid peroxidation. Under control conditions, BAS1 is present at similar levels both in wild-type (WT) and transgenic plants. Under drought and methyl viologen treatment, CDSP32-lacking plants display, compared with WT, an increased proportion of BAS1 monomer corresponding to an overoxidized form of the protein. Leaf discs from transgenic plants treated with methyl viologen exhibit earlier degradation of BAS1 than WT plants do. Using several approaches, i.e. a probe emitting fluorescence when reacting with peroxides, high-performance liquid chromatography determination of lipid hydroxy fatty acid content, and measurement of chlorophyll thermoluminescence, we show a higher lipid peroxidation level under methyl viologen treatment in thylakoids from CDSP32-lacking plants compared with WT. These data show that CDSP32 is a critical component in the defense system against lipid peroxidation in photosynthetic membranes, likely as a physiological electron donor to the BAS1 peroxiredoxin.

L6 ANSWER 15 OF 27 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2003338268 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12870673
TITLE: Upregulation of redox-regulating protein, thioredoxin, in endomyocardial biopsy samples of patients with myocarditis and cardiomyopathies.
AUTHOR: Nimata Masaomi; Kishimoto Chiharu; Shioji Keisuke; Ishizaki Katsumi; Kitaguchi Shoji; Hashimoto Tetsuo; Nagata Norikazu; Kawai Chuichi
CORPORATE SOURCE: Department of Cardiovascular Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan.
SOURCE: Molecular and cellular biochemistry, (2003 Jun) Vol. 248,

No. 1-2, pp. 193-6.
Journal code: 0364456. ISSN: 0300-8177.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200404
ENTRY DATE: Entered STN: 22 Jul 2003
Last Updated on STN: 20 Apr 2004
Entered Medline: 19 Apr 2004

AB An important role of redox regulation in myocardial diseases and heart failure has been postulated. Thioredoxin (TRX) is a redox-regulating protein. Recent studies indicated a possible association between plasma TRX concentrations and the severity of heart failure. Accordingly, we investigated the myocardial expression of TRX in patients with myocarditis and cardiomyopathies. Four cases of hypertrophic cardiomyopathy (HCM), 10 of dilated cardiomyopathy (DCM), 6 of myocarditis, and 5 of controls were studied. Right and left ventricular endomyocardial biopsy samples were obtained at the diagnostic cardiac catheterization. The samples were processed for immunohistological staining for TRX, which was done by the indirect immunoperoxidase technique. 8-hydoxy-2'-deoxyguanosine (8-OHdG), one of the major DNA base-modified products, was also detected for an established marker for oxidative stress. TRX immunoreactivity was none or trivial in control specimens. Positive TRX staining was found in 6 cases; 3 in active myocarditis and 3 in DCM. The positive staining was found in infiltrating cells and damaged myocytes in the perinecrotic lesions. Damaged myocytes were also positive for 8-OHdG All the 3 cases of DCM positive for TRX stain showed severe left ventricular hypertrophy on electrocardiogram and highly elevated left ventricular end-diastolic pressure (> 24 mmHg), suggesting the overload of oxidative stress by hemodynamic impairment. Myocardial TRX was upregulated in myocarditis and cardiomyopathies with active necrotic stage associated with DNA damage, which may reflect the oxidative stress overload in hemodynamically uncontrolled status.

L6 ANSWER 16 OF 27 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2003338267 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12870672
TITLE: Temocapril treatment ameliorates autoimmune myocarditis associated with enhanced cardiomyocyte thioredoxin expression.
AUTHOR: Yuan Zuyi; Kishimoto Chiharu; Shioji Keisuke; Nakamura Hajime; Yodoi Junji; Sasayama Shigekake
CORPORATE SOURCE: Department of Cardiovascular Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan.
SOURCE: Molecular and cellular biochemistry, (2003 Jun) Vol. 248, No. 1-2, pp. 185-92.
Journal code: 0364456. ISSN: 0300-8177.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200404
ENTRY DATE: Entered STN: 22 Jul 2003
Last Updated on STN: 20 Apr 2004
Entered Medline: 19 Apr 2004

AB Thioredoxin (TRX) is a redox regulatory protein that protects cells from various stresses. Angiotensin-converting enzyme (ACE) inhibitor was reported to enhance endogenous antioxidant enzyme activities. This study was carried out to investigate whether temocapril, a novel non-sulfhydryl containing ACE inhibitor, reduces the severity of myocarditis via redox regulation mechanisms involving TRX. Western blot showed that temocapril enhanced cytosolic redox regulatory protein TRX

expression, but neither mitochondrial TRX2 nor antioxidant enzymes, such as copper-zinc superoxide dismutase (Cu/Zn-SOD) or manganese superoxide dismutase (Mn-SOD) expression, was increased by the preconditioning treatment. In rats with experimental autoimmune myocarditis (EAM), the protein carbonyl content, a marker of cellular protein oxidation, was increased accompanied with enhanced TRX expression. An immunohistochemical study showed that TRX stain was enhanced in infiltrating inflammatory cells and in damaged myocytes. The severity of the myocarditis and the protein carbonyl contents were less increased in temocapril treatment (10 mg/kg/day, orally) from day 1 to day 21 in which TRX was up regulated when the inflammation started, but not in temocapril treatment from day 15-21 in which TRX was not up-regulated when the inflammation started. The results suggest that TRX and the redox state modified by TRX may play a crucial role in the pathophysiology of EAM. Temocapril ameliorates myocarditis associated with inducing TRX increase in a preconditioning manner, although the mechanism of TRX induction by temocapril remains to be elucidated.

L6 ANSWER 17 OF 27 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-00726 BIOTECHDS
TITLE: Novel polypeptide having cytotoxic activity obtainable from Aplysia, useful for destroying tumors, for identifying novel targets for the development of anti-tumor agents, and as specific ion channel modulators;
vector-mediated recombinant protein gene transfer and expression in host cell for disease prevention, diagnosis and therapy
AUTHOR: BUTZKE D; MACHUY N; RUDEL T; MEYER T F
PATENT ASSIGNEE: MAX PLANCK GES FOERDERUNG WISSENSCHAFTEN
PATENT INFO: WO 2002031144 18 Apr 2002
APPLICATION INFO: WO 2001-EP11837 12 Oct 2001
PRIORITY INFO: EP 2000-122466 13 Oct 2000; EP 2000-122466 13 Oct 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-537205 [57]
AB DERWENT ABSTRACT:
NOVELTY - A polypeptide (I) having cytotoxic activity obtainable from the sea hare Aplysia and having a molecular mass of about 60 +/- 5 kDa as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or its fragment, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a nucleic acid (II) encoding (I); (2) a recombinant cell (III) transformed or transfected with (II); (3) an antibody (IV) directed against (I); (4) a pharmaceutical composition (V) comprising as an active agent (I), (II), (III) or (IV); (5) a target substance (VI) detectable using (I); and (6) a pharmaceutical composition (VII) comprising as an active agent (VI) or a modulator, e.g. inhibitor or activator of (VI).

BIOTECHNOLOGY - Preparation: (I) is prepared by standard recombinant techniques. Preferred Polypeptide: (I) has cytotoxic activity towards the human T cell line Jurkat. (I) is resistant against proteolytic digestion and incubation with N-acetyl neuraminic acid. (I) is an ion channel modulator. Preferred Nucleic Acid: (II) comprises a sequence of 442 or 462 base pairs fully defined in the specification, or its polypeptide coding portion or complement, a nucleotide sequence corresponding to the above mentioned sequence within the scope of degeneracy of the genetic code, or its complement, and/or a nucleotide sequence hybridizing under stringent conditions with the above mentioned sequences. (II) is operatively linked to an expression control sequence. (II) is a recombinant vector. Preferred Substance: (VI) is selected from 23 substances such as thioredoxin peroxidase 2 or 60S ribosomal protein PO.

ACTIVITY - Antitumor; Nootropic; Neuroprotective; Anti-parkinsonian; Antiarteriosclerotic; Cerebroprotective; Cardiant.

MECHANISM OF ACTION - Ion channel modulator (claimed). No supporting

data is given.

USE - (I) is useful for determining targets of (I) by contacting (I) with a biological sample, determining interactions between (I) and a substance contained in the sample, and identifying and/or characterizing the substance as a target substance. The determining step comprises detecting target substances which are present in a modified form in a sample which has been contacted with (I) compared to a control sample. The target substance is a direct or indirect cellular target of (I). (I) or (II) are useful for the manufacture of a cytotoxic agent against apoptosis-resistant cells, where the agent is useful for diagnosis, prevention, treatment or disorders associated with dysfunctions in (VI), such as dysfunctions of GAP-SH3 binding protein, factors for generating or detoxifying reactive oxygen species (ROS) and factors for blocking and/or by-passing of caspases. (V) is useful for tumor therapy. (VI) is useful for the identification of new pharmaceutical agents, e.g., by a screening procedure (claimed). (I) is useful for destroying tumors and/or selectively killing cells in tissues, for identifying novel targets for the development of pharmaceutical agents, preferably anti-tumor agents, and as specific ion channel modulators, e.g., blockers or openers for therapy, diagnostic or research. (V) is useful for diagnosis and therapy of hyperproliferative diseases, preferably tumors, e.g., leukemia, carcinoma, sarcoma and melanoma. (VI) is useful for development of drugs for treatment of degenerative diseases such as Alzheimer's disease, Parkinson's disease, arteriosclerosis, heart diseases, stroke and vascular diseases.

EXAMPLE - Purification and characterization of a cytotoxic activity from Aplysia punctata ink involved harvesting the ink by injecting 380 mM MgCl2 into the body cavity and smooth massage of the sea hares. The ink was applied to a gel filtration Superdex HR200 column which was previously equilibrated with phosphate buffered saline (PBS). All fractions were analyzed for their cytotoxic activity and the active fractions were pooled. The buffer of the pooled active fractions from the gel filtration were adjusted to 20 mM Tris, pH 8 and the samples were then applied to a MonoQ anion exchange column. Bound proteins were eluted using a linear NaCl gradient from 0-1000 mM NaCl. One dimensional gel electrophoresis was performed. The protein was blotted on a polyvinylidene difluoride (PVDF) membrane. The proteins were separated by a small gel two-dimensional technique. Preparation gels were stained with Coomassie Brilliant Blue R-250. PVDF membranes were stained with Coomassie Brilliant Blue R-250. Analytical gels were stained with silver nitrate. The Coomassie Brilliant Blue R-250 stained single gel spots were excised. Equilibration of the gel pieces was performed. Subsequently, shrinkage of the gel spots was performed. After removing of the solution first equilibration, then shrinkage and finally equilibration were performed again. Then the gel spots were dried. Next, 0.1 microg of trypsin solved in 1 microl 50 mM acetic acid and 19 microl 50 mM ammonium bicarbonate, pH 7.8 was added. After incubation, the supernatant was removed and the gel pieces were washed and again the supernatant was removed. The combined supernatants were evaporated and solved in 2 microl 0.5% aqueous TFA/acetonitrile for the mass spectrometrical analysis. Peptide mass fingerprinting and sequencing was performed. (87 pages)

L6 ANSWER 18 OF 27 MEDLINE on STN DUPLICATE 6

ACCESSION NUMBER: 2002378261 MEDLINE

DOCUMENT NUMBER: PubMed ID: 12123771

TITLE: Temocapril treatment ameliorates autoimmune myocarditis associated with enhanced cardiomyocyte thioredoxin expression.

AUTHOR: Yuan Zuyi; Kishimoto Chiharu; Shioji Keisuke; Nakamura Hajime; Yodoi Junji; Sasayama Shigekake

CORPORATE SOURCE: Department of Cardiovascular Medicine, Graduate School of Medicine, Kyoto University, 54 Kawaracho, Shogoin, Sakyo-ku, Kyoto, 606-8507, Japan.

SOURCE: Cardiovascular research, (2002 Aug 1) Vol. 55, No. 2, pp. 320-8.
Journal code: 0077427. ISSN: 0008-6363.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200210
ENTRY DATE: Entered STN: 19 Jul 2002
Last Updated on STN: 10 Oct 2002
Entered Medline: 9 Oct 2002

AB OBJECTIVE: Thioredoxin (TRX) is a redox regulatory protein that protects cells from various stresses. Angiotensin-converting enzyme (ACE) inhibitor was reported to enhance endogenous antioxidant enzyme activities. This study was carried out to investigate whether temocapril, a novel non-sulfhydryl-containing ACE inhibitor, reduces the severity of myocarditis via redox regulation mechanisms involving TRX. METHODS AND RESULTS: In normal rat myocytes in vitro and in vivo, Western blot showed that temocapril enhanced cytosolic redox regulatory protein TRX expression, but that neither mitochondrial TRX2 nor antioxidant enzymes, such as copper-zinc superoxide dismutase (Cu/Zn-SOD) or manganese superoxide dismutase (Mn-SOD) expression, was up-regulated by the preconditioning treatment. In rats with experimental autoimmune myocarditis (EAM), the severity of myocarditis and the protein carbonyl contents were less increased in temocapril treatment (10 mg/kg/day, orally) from day 1 to day 21, but not in temocapril treatment from day 15 to day 21. An immunohistochemical study showed that TRX stain was enhanced in infiltrating inflammatory cells and in damaged myocytes. Considering the characteristics of this model that myocardial inflammation begins around day 15 and increases until day 21, temocapril treatment for 3 weeks might be thought of as a preconditioning treatment. CONCLUSIONS: The results suggest that TRX and the redox state modified by TRX may play a crucial role in the pathophysiology of EAM. Temocapril ameliorates myocarditis associated with inducing TRX up-regulation in a preconditioning manner, although the mechanism of TRX up-regulation by temocapril remains to be elucidated.

L6 ANSWER 19 OF 27 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-07519 BIOTECHDS
TITLE: Novel apoptosis-associated and/or -modified protein useful as target for the diagnosis, prevention or treatment of apoptosis-associated diseases particularly neurodegenerative diseases;
protein DNA fingerprinting, and mass spectroscopy useful for protein chip construction and high throughput screening
AUTHOR: RUDEL T; THIEDE B; MACHUY N
PATENT ASSIGNEE: MAX PLANCK GES FOERDERUNG WISSENSCHAFTEN
PATENT INFO: WO 2001096873 20 Dec 2001
APPLICATION INFO: WO 2000-EP6780 16 Jun 2000
PRIORITY INFO: EP 2000-125013 16 Nov 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-098088 [13]

AB DERWENT ABSTRACT:
NOVELTY - An apoptosis-associated and/or -modified protein (I) selected from GAP SH3 binding protein, HCD2 and AOP-1 or its proteolytic fragments, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) characterizing and/or identifying (M1) apoptosis-modified proteins, comprising: (a) providing a first extract (E1) and a second extract (E2) comprising soluble proteins, where E1 is from a cell without apoptosis induction and E2 is from a cell after apoptosis induction; (b) separating E1 and E2 by two-dimensional gel

electrophoresis, where first and second proteome patterns each comprising a number of protein species were obtained; (c) comparing the first and second proteome patterns; and (d) characterizing and/or identifying apoptosis-modified protein species; (2) proteome from an apoptotic T-cell or its compartment, consisting of a pattern of individual proteins obtained by the method of (1); (3) inhibiting caspase cleavage of apoptosis-associated and/or -modified proteins, so that the caspase cleavage site is modified to avoid cleavage; and (4) use of a caspase cleavage site to design and/or screen for substances that inhibit or modulate caspase cleavage of proteins containing such cleavage sites.

WIDER DISCLOSURE - Disclosed as new are the following: (1) proteins translocated from one cellular compartment such as nucleus, cytosol, mitochondria or membrane to another; (2) nucleic acid molecules encoding (I); and (3) substances capable of modulating the characteristics of the proteins or nucleic acids.

BIOTECHNOLOGY - Preferred Method: In M1, after apoptosis induction no synthesis of new proteins has been allowed. The protein biosynthesis has been blocked by an inhibitor, and apoptosis induction has been carried out for a period of time which is too short to allow synthesis of new proteins. The two-dimensional gel electrophoresis comprises separation in a first dimension according to the isoelectric point and separation in a second dimension according to the size. The apoptosis-modified protein species are selected from protein species which are located at different positions on the two-dimensional gels from E1 and E2, and/or have a different intensity on the two-dimensional gels from E1 and E2. The protein species are characterized by protein fingerprinting, and the peptides are characterized by mass spectrometry and/or at least partial sequencing. The cell is a mammalian cell e.g. human T-cell such as T-cell line Jurkat E6 (ATCC TIB 152). The apoptosis is induced by an anti-Fas antibody or by treatment with cis-platin. M1 further involves determining the apoptosis-modified proteins are present in subjects suffering from apoptosis-associated diseases. Preferred Protein: (I) is selected from heterogeneous nuclear ribonucleoproteins, splicing factors, translation factors, structural proteins, signal transduction proteins, chromatin associated proteins, transcription factors, proteasome subunits, mitochondrial proteins, nucleophosmin, SYT interacting protein SIP, PA1-G, CRHSP-24, HCD2, GMP synthase, FUSe binding protein 1, HDGF, PFC6D, KPF1, KNFE3 having the partial sequence TPGT (F/Mox)E, alpha-NAC, ARDH, cargo selection protein, DAZ associated protein 1, DEAD box protein retinoblastoma, dihydrofolate reductase, hydroxyacyl-CoA dehydrogenase/3-ketoactyl-CoA thiolase/enoyl-CoA hydratase, ER-60, HCA56, Hsp-105, IGF-II mRNA binding protein 1, IGF-II mRNA binding protein 3, lactate dehydrogenase A, NS-associated protein, RAD 21, RAD 23 homolog B, T-complex protein beta subunit, thioredoxin-like protein, an unnamed protein (NCBI 7020309), and c-Abl, or a partial sequence derived from them by substitution and/or deletion of one or more amino acids. The proteome contains proteins that are given in the specification or at least their part. The caspase cleavage site is contained in or combined with a reporter protein, and is characterized by the amino acid sequence XXXD, where X denotes any amino acid.

ACTIVITY - Cytostatic; immunosuppressive; nootropic; neuroprotective; virucide; anti-HIV (human immunodeficiency virus); cardiant; vasotropic; antiparkinsonian; cerebroprotective; antiarthritic. No biological data is given.

MECHANISM OF ACTION - Apoptosis modulator.

USE - (I) is useful as target for the diagnosis, prevention or treatment of apoptosis-associated diseases, for the manufacture of a pharmaceutical agent and for identifying apoptosis modulators. The proteome is useful as a target for the diagnosis, prevention or treatment of apoptosis-associated diseases, and in a method for identifying apoptosis modulators. (All claimed). (I) is useful for treatment of hyperproliferative or degenerative diseases including cancers, autoimmune

diseases, Alzheimer's disease, viral infections such as acquired immunodeficiency syndrome (AIDS) and vascular diseases such as myocardial infarction. (I) is also useful for treating and/or preventing diseases related to oxidative stress like ischemic stroke, arthritis, heart failure, Parkinson's disease and amyotrophic lateral sclerosis. (I) is also useful to develop modification-specific diagnostic tools. (I) is also useful for developing protein chips or other solid phase screening devices for high throughput screens, and also as a diagnostic tool to screen for caspase activity, and/or to determine the effectivity of caspase cleavage inhibiting and/or modulating substances.

EXAMPLE - Apoptosis was induced in Jurkat-T cells by treatment with an anti-Fas antibody for six hours. 2-dimensional electrophoresis (2-DE) gels were produced after lysis of the cells and separation of the proteins. 2000 spots were resolved and detected by silver staining. Ten 2-DE gels of Jurkat T-cells protein patterns of apoptosis-induced cells and control cells were found to be highly reproducible. In Fas-induced Jurkat T-cells 24 additional spots and in untreated Jurkat T-cells 21 additional sports were observed. Coomassie stained 2-DE gels were used for the identification by mass spectrometry. The proteins of the total cell lysate were identified within 21 spots by peptide mass fingerprinting after in-gel digestion with trypsin, elution of the generated peptides and analysis by DE-MALDI-MS (Matrix-assisted laser desorption ionization-mass spectrometry). In the total cell lysate, 10 additional proteins were identified after Fas induction, whereas 6 proteins disappeared. Four proteins (hnRNP A2/B1, hnRNP Ca/C2, p54nrb and Rho GDI 2) were found at different spot positions in negative- and positive Fas cells, whereas the other proteins were only identified at one condition. The molecular mass of protein spots in 2-DE gels were determined with an accuracy of 10 %. The identified proteins in negative Fas gels displayed the theoretical mass of the corresponding protein. Five of the apoptosis-modified positive Fas proteins showed a significant decreased mass, whereas the remaining three proteins hnRNP C1/C2, p54nrb and splicing factor STp30c retained the expected theoretical mass. The negative Fas spot of p54nrb showed an increased mass of 3.6 kDa in comparison to the positive Fas spot of the same protein. The negative Fas spot of the hnRNP C1/C2 spots displayed an increased mass of 1 kDa and decreased pI of 0.4 in comparison to the positive Fas spots. The mass and pI of the splicing factor SRp30c in Fas-positive Jurkat T-cells showed the theoretical values. The results indicated that predominantly cleavage events had occurred within the identified proteins during the apoptotic process. The identified protein shared similarities concerning function and motifs. The hnRNPs and the splicing factors were involved in the splicing process. 8 proteins contain the RNP-motif and 7 proteins include an aspartic acid/glutamic acid rich domain. Interaction with protein kinase CK2 was already identified for hnRNP A2/B1, hnRNP C1/C2, nucleolin and the transcription factor BTF3.(86 pages)

L6 ANSWER 20 OF 27 MEDLINE on STN DUPLICATE 7

ACCESSION NUMBER: 2001252962 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11295360
TITLE: Variants of peroxiredoxins expression in response to hydroperoxide stress.
AUTHOR: Mitsumoto A; Takanezawa Y; Okawa K; Iwamatsu A; Nakagawa Y
CORPORATE SOURCE: School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan.
SOURCE: Free radical biology & medicine, (2001 Mar 15) Vol. 30, No. 6, pp. 625-35.
Journal code: 8709159. ISSN: 0891-5849.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106

ENTRY DATE: Entered STN: 11 Jun 2001
Last Updated on STN: 11 Jun 2001
Entered Medline: 7 Jun 2001

AB We examined patterns of the proteins that were expressed in human umbilical vein endothelial cells (HUVEC) in response to oxidative stress by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). When HUVEC were exposed to H2O2 at 100 microM for 60 min, the intensities of eight spots increased and those of eight spots decreased on 2D gels, as compared with control gels, after staining with silver. These changes were also observed after exposure of cells to hydroperoxides such as cumene hydroperoxide and tert-butyl hydroperoxide, but not after exposure to other reagents that induce oxidative stress such as S-alkylating compounds, nitric oxide, and salts of heavy metals. Therefore, these proteins were designated hydroperoxide responsive proteins (HPRPs). Microsequencing analysis revealed that these HPRPs corresponded to at least six pairs of proteins. Of these, four pairs of HPRPs were thioredoxin peroxidase I (TPx I), TPx II, TPx III, and the product of human ORF06, all of which belong to the peroxiredoxin (Prx) family and all of which are involved in the elimination of hydroperoxides. The other two pairs corresponded to heat shock protein 27 (HSP27) and glyceraldehyde-3-phosphate dehydrogenase (G3PDH), respectively. The variants that appeared in response to hydroperoxides had molecular masses similar to the respective native forms, but their pI values were lower by 0.2-0.3 pH units than those of the corresponding native proteins. These variants were detected on 2D gels after cells had been exposed to hydroperoxides in the presence of an inhibitor of protein synthesis. All variants were generated within 30 min of exposure to 100 microM H2O2. The variants of TPx I and TPx II appeared within 2 min of the addition of H2O2 to the culture medium. The HPRPs returned to their respective native forms after the removal of stress. Our results indicated that at least six proteins were structurally modified in response to hydroperoxides. Analysis by 2D-PAGE of 32P-labeled proteins revealed that the variant of HSP27 was its phosphorylated form while the other HPRPs were not modified by phosphorylation. Taken together, the results suggest that 2D-PAGE can reveal initial responses to hydroperoxide stress at the level of protein modification. Moreover, it is possible that the variants of four types of Prx might reflect intermediate states in the process of hydroperoxide elimination.

L6 ANSWER 21 OF 27 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2000281229 EMBASE
TITLE: Identification of foetal brain proteins by two-dimensional gel electrophoresis and mass spectrometry. Comparison of samples from individuals with or without chromosome 21 trisomy.
AUTHOR: Oppermann M.; Cols N.; Nyman T.; Helin J.; Saarinen J.; Byman I.; Toran N.; Alaiya A.A.; Bergman T.; Kalkkinen N.; Gonzalez-Duarte R.; Jornvall H.
CORPORATE SOURCE: H. Jornvall, Dept. of Med. Biochem./Biophysics, Karolinska Institutet, SE-17177 Stockholm, Sweden. Hans.Jornvall@mbb.ki.se
SOURCE: European Journal of Biochemistry, (2000) Vol. 267, No. 15, pp. 4713-4719. .
Refs: 26
ISSN: 0014-2956 CODEN: EJBCAI
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 021 Developmental Biology and Teratology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 31 Aug 2000
Last Updated on STN: 31 Aug 2000

AB Protein expression in foetal brain with or without chromosome 21 trisomy

(Down's syndrome) was analyzed by two-dimensional gel electrophoresis and mass spectrometry. Data generated by in-gel digestion and matrix-assisted laser desorption/ionization mass spectrometry allowed identification of 40 proteins. Most of these are common to syndrome and healthy subjects and represent different types of protein. However, a few proteins, identified as truncated structural proteins (tubulin, actin), were present in part of the trisomy samples but absent from the controls. This is interpreted to indicate increased proteolysis in the syndrome samples but could also reflect some altered expression or processing. Independent of the apparently increased proteolysis in the syndrome samples, and in spite of the use of total brain tissues, the results show that two-dimensional protein separation patterns are largely similar between the syndrome and control samples upon silver-staining, but that differences associated with structural components can be detected and identified.

L6 ANSWER 22 OF 27 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 1999384395 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10452910
TITLE: Expression of thioredoxin and thioredoxin reductase in placentae of pregnant mice exposed to lipopolysaccharide.
AUTHOR: Ejima K; Koji T; Nanri H; Kashimura M; Ikeda M
CORPORATE SOURCE: Department of Health Development, University of Occupational and Environmental Health, Kitakyushu, 807-8555, Japan.
SOURCE: Placenta, (1999 Sep) Vol. 20, No. 7, pp. 561-6. Journal code: 8006349. ISSN: 0143-4004.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 12 Oct 1999
Last Updated on STN: 30 Oct 2002
Entered Medline: 27 Sep 1999

AB We have previously shown that thioredoxin and thioredoxin reductase were immunohistochemically localized in cytotrophoblasts, decidua and stromal cells in the stem villi of human placenta and that the addition of exogenous thioredoxin and thioredoxin reductase to mitochondrial fractions from human placenta displayed a protective effect on fumarase activity against oxidative stress. In this study, to investigate further the roles of thioredoxin and thioredoxin reductase in protecting pregnancy against oxidative stress, we examined the effect of lipopolysaccharide (LPS), which induces a variety of cytokines and produces radical oxygen species, on the expression of thioredoxin and thioredoxin reductase in mouse placenta. We focused on the placental protective effect in the second trimester, when the onset of placental dysfunction might occasionally lead to a critical state for the fetus. Thus we analysed placentae from mice on day 13 of pregnancy at various time points after they were injected with LPS (50 microg/kg i.p.) or saline as a control. The expressions of thioredoxin and thioredoxin reductase were evaluated by Western blotting and immunohistochemistry. Western blot analysis revealed that LPS approximately quadrupled the expression of both thioredoxin and thioredoxin reductase in the placentae of pregnant mice. When both proteins were localized immunohistochemically, it was found that the decidua and the diploid trophoblasts in the basal zone were intensively stained. Furthermore, the expression of 4-hydroxy-2-nonenal (HNE)-modified proteins, which are markers of oxidative stress, was enhanced in placenta by LPS. Our study suggests that the induced thioredoxin and thioredoxin reductase might protect the placenta from the stress induced by LPS.

L6 ANSWER 23 OF 27 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
DUPLICATE 9
ACCESSION NUMBER: 1998-09126 BIOTECHDS
TITLE: Production of polypeptides as inclusion bodies; recombinant protein preparation by plasmid pTrc99A or plasmid pTrxfus vector-mediated thioredoxin expression in Escherichia coli inclusion body
AUTHOR: Chatterjee D; Longo M; Flynn E; Oberfelder R
PATENT ASSIGNEE: Life-Technol.
LOCATION: Rockville, MD, USA.
PATENT INFO: WO 9830684 16 Jul 1998
APPLICATION INFO: WO 1998-US492 8 Jan 1998
PRIORITY INFO: US 1997-34658 8 Jan 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1998-399134 [34]
AB A new method for the preparation of a protein in the form of an inclusion body involves: obtaining a host cell (e.g. Escherichia coli) containing a first DNA sequence encoding the protein linked to a second DNA sequence encoding an inclusion partner (thioredoxin or modified thioredoxin), forming a gene fusion construct (plasmid pTrcpr1-monomer or pTrxA-concat); and culturing the cell to favor production of the protein as inclusion bodies. Also new are: vector plasmid pTrc99A and plasmid pTrxfus containing the construct; a host cell containing the vector; making a protein mol.weight ladder composition by obtaining one or more DNA sequences encoding proteins of different mol.weight values, transforming host cells with the DNA, culturing the cells to favor production of each protein, and isolating each protein; and making one or more stained proteins by incubating the proteins with one or more protein-binding dyes under incubation conditions to complex the proteins with the dyes. The methods may be used to prepare a fragment of the gene-32 protein of phage T4, a fragment of KpnI-methylase or a fragment of E. coli Dead-Box protein or thioredoxin. (84pp)

L6 ANSWER 24 OF 27 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 96378635 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8784198
TITLE: The function and properties of the iron-sulfur center in spinach ferredoxin: thioredoxin reductase: a new biological role for iron-sulfur clusters.
AUTHOR: Staples C R; Ameyibor E; Fu W; Gardet-Salvi L; Stritt-Etter A L; Schurmann P; Knaff D B; Johnson M K
CORPORATE SOURCE: Department of Chemistry, University of Georgia, Athens 30602, USA.
CONTRACT NUMBER: R01-GM51962 (NIGMS)
SOURCE: Biochemistry, (1996 Sep 3) Vol. 35, No. 35, pp. 11425-34. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199610
ENTRY DATE: Entered STN: 25 Oct 1996
Last Updated on STN: 3 Mar 2000
Entered Medline: 11 Oct 1996
AB Thioredoxin reduction in chloroplasts is catalyzed by a unique class of disulfide reductases which use a [2Fe-2S]2+/+ ferredoxin as the electron donor and contain an Fe-S cluster as the sole prosthetic group in addition to the active-site disulfide. The nature, properties, and function of the Fe-S cluster in spinach ferredoxin:thioredoxin reductase (FTR) have been investigated by the combination of UV/visible absorption, variable-temperature magnetic circular dichroism (MCD), EPR,

and resonance Raman (RR) spectroscopies. The results indicate the presence of an S = 0 [4Fe-4S]2+ cluster with complete cysteinyl-S coordination that cannot be reduced at potentials down to -650 mV, but can be oxidized by ferricyanide to an S = 1/2 [4Fe-4S]3+ state (g = 2.09, 2.04, 2.02). The midpoint potential for the [4Fe-4S]3+/2+ couple is estimated to be +420 mV (versus NHE). These results argue against a role for the cluster in mediating electron transport from ferredoxin (Em = -420 mV) to the active-site disulfide (Em = -230 mV, n = 2). An alternative role for the cluster in stabilizing the one-electron-reduced intermediate is suggested by parallel spectroscopic studies of a modified form of the enzyme in which one of the cysteines of the active-site dithiol has been alkylated with N-ethylmaleimide (NEM). NEM-modified FTR is paramagnetic as prepared and exhibits a slow relaxing, S = 1/2 EPR signal, g = 2.11, 2.00, 1.98, that is observable without significant broadening up to 150 K. While the relaxation properties are characteristic of a radical species, MCD, RR, and absorption studies indicate at least partial cluster oxidation to the [4Fe-4S]3+ state. Dye-mediated EPR redox titrations indicate a midpoint potential of -210 mV for the one-electron reduction to a diamagnetic state. By analogy with the properties of the ferricyanide-oxidized [4Fe-4S] cluster in Azotobacter vinelandii 7Fe ferredoxin [Hu, Z., Jollie, D., Burgess, B. K., Stephens, P. J., & Munck, E. (1994) Biochemistry 33, 14475-14485], the spectroscopic and redox properties of NEM-modified FTR are interpreted in terms of a [4Fe-4S]2+ cluster covalently attached through a cluster sulfide to a cysteine-based thiyl radical formed on one of the active-site thiols. A mechanistic scheme for FTR is proposed with similarities to that established for the well-characterized NAD(P)H-dependent flavin-containing disulfide oxidoreductases, but involving sequential one-electron redox processes with the role of the [4Fe-4S]2+ cluster being to stabilize the thiyl radical formed by the initial one-electron reduction of the active-site disulfide. The results indicate a new biological role for Fe-S clusters involving both the stabilization of a thiyl radical intermediate and cluster site-specific chemistry involving a bridging sulfide.

L6 ANSWER 25 OF 27 MEDLINE on STN
ACCESSION NUMBER: 93018290 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1328424
TITLE: Evaluation of environmental factors affecting embryo development in vitro.
AUTHOR: Noda Y
CORPORATE SOURCE: Department of Gynecology and Obstetrics, Faculty of Medicine, Kyoto University.
SOURCE: Nippon Sanka Fujinka Gakkai zasshi, (1992 Aug) Vol. 44, No. 8, pp. 960-70.
Journal code: 7505749. ISSN: 0300-9165.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Japanese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199211
ENTRY DATE: Entered STN: 22 Jan 1993
Last Updated on STN: 22 Jan 1993
Entered Medline: 17 Nov 1992

AB Human in vitro fertilization and embryo transfer (IVF-ET) became an indispensable modality for treating infertile patients. The principle of this method is simple: that is, recovery of gametes from the gonads of men and women and transfer of the embryos into the uterus. This method can be expected, therefore, to be applied to many patients with a variety of causes of infertility. Unfortunately, the success rates are not satisfactory in the majority of clinics in the 14 years since the first report of a test tube in 1978. In view of improving the success rate, one major issue is the protocol used for ovulation induction, which may

influence the quality of eggs as well as the environmental conditions in the endometrium at the time of embryo replacement. Another major issue should be the technique for embryo culture because, in general, mammalian embryos, including humans', are known to exhibit developmental retardation in vitro. In a significant number of embryos, cleavage is arrested at the first or second cell cycle when cultured under the conventional culture conditions. This phenomenon in rodents is known as "block to development in vitro" or "two-cell block in vitro". Recently, the mouse two-cell block was found to be attenuated by the addition of superoxide dismutase (SOD) to the culture medium. SOD is the enzyme that catalyzes the dismutation reaction of superoxide anion radicals: 2O2- + 2H(+)----H2O2 + O2. This suggests that developmental retardation in vitro may be related to the potential oxygen toxicity that embryos encounter in vitro. Following to this finding, a variety of culture conditions have been found to attenuate blocking phenomenon and to increase blastulation rate in the mouse embryos. By the addition of chemicals to the culture medium such as L-Cysteine, L-Ascorbic acid, EDTA, DTPA or thiredoxine, blastulation rates could be increased overcoming blocking phenomenon. From these findings, it seemed possible to hypothesize that developmental retardation is caused by the oxidative stress that embryos encounter in vitro. Oxidative stress is defined as an increased intracellular concentration of the active oxygen species in a stead-state condition. To make the hypothesis validated, intracellular generation of active oxygen species was measured by using DCHF-DA, a fluorescence dye precursor. The results showed that the fluorescent emissions of embryos were lowest in embryos cultured under 5% O2 and highest under 40% O2. L-Cysteine and thioredoxin, both of which have been shown to promote the embryo development, decreased the fluorescence emissions of embryos.(ABSTRACT TRUNCATED AT 400 WORDS)

L6 ANSWER 26 OF 27 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1989:106625 HCAPLUS
DOCUMENT NUMBER: 110:106625
TITLE: A switching device with protein and Langmuir-Blodgett redox films
INVENTOR(S): Isoda, Satoru; Kamiyama, Tomotsugu; Kawakubo, Hiroaki
PATENT ASSIGNEE(S): Mitsubishi Electric Corp., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 7 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 63237562	A2	19881004	JP 1987-73350	19870326
JP 06080814	B4	19941012		

PRIORITY APPLN. INFO.: JP 1987-73350 19870326

AB The device, which shows a transistor or switching property, comprises the following: (1) a 1st film of a 1st redox material; (2) a 2nd film, which is made of a 2nd redox material having a redox potential different from the 1st, on the 1st film; (3) a 3rd film, which is made of a 3rd redox material having a redox potential different from the 2nd; (4) 1st and 3rd electrodes for the 1st and 3rd films; and (5) a 2nd electrode which elec. affects the 2nd film. The 1st, 2nd, or 3rd film comprises a redox (pseudo)protein, and 1 of the remaining 2 films comprises a Langmuir-Blodgett film of, or the electrode modified with, an organic mol. The remaining film comprises the protein, Langmuir-Blodgett, or chemical-modified film. Optionally, the protein may comprise a nonheme-Fe-S protein, cytochrome c, cytochrome b, cytochrome a, flavodoxin, plastocyanin, or thioredoxin, and the organic mol. may comprise a viologen, flavin, thionin, methylene blue, methylcapryl blue, gallocyanin, indophenol, indigo, phenosofranine, Neutral Red, or toluidine

blue.

L6 ANSWER 27 OF 27 MEDLINE on STN
ACCESSION NUMBER: 87000555 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3019388
TITLE: Alteration of intramolecular disulfides in insulin receptor/kinase by insulin and dithiothreitol: insulin potentiates the apparent dithiothreitol-dependent subunit reduction of insulin receptor.
AUTHOR: Wilden P A; Boyle T R; Swanson M L; Sweet L J; Pessin J E
CONTRACT NUMBER: AM07018 (NIADDK)
AM25295 (NIADDK)
AM33823 (NIADDK)
SOURCE: Biochemistry, (1986 Jul 29) Vol. 25, No. 15, pp. 4381-8.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198611
ENTRY DATE: Entered STN: 2 Mar 1990
Last Updated on STN: 3 Mar 2000
Entered Medline: 7 Nov 1986

AB Dithiothreitol (DTT) was observed to increase both beta-subunit autophosphorylation and exogenous substrate phosphorylation of the insulin receptor in the absence of insulin. The natural protein reducing agent thioredoxin was also observed to increase the insulin receptor beta-subunit autophosphorylation. The activation of the insulin receptor/kinase by both DTT and thioredoxin was found to be additive with that of insulin. Further, the increase in the insulin receptor beta-subunit autophosphorylation in the presence of DTT and insulin was demonstrated to be due to an increase in the initial rate of autophosphorylation without alteration in the extent of phosphorylation. Similarly, the increase in the exogenous substrate phosphorylation was due to an increase in the Vmax of phosphorylation without significant effect on the apparent Km of substrate binding. In the presence of relatively low concentrations of DTT, insulin was found to potentiate the apparent insulin receptor subunit reduction of the native alpha 2 beta 2 heterotetrameric complex into alpha beta heterodimers, when observed by silver staining of sodium dodecyl sulfate-polyacrylamide gels. N-[3H]Ethylmaleimide ([3H]NEM) labeling in the absence of DTT pretreatment demonstrated that only the beta subunit had accessible sulfhydryl group(s). However, treatment of insulin receptors with DTT increased the amount of [3H]NEM labeling in the beta subunit as well as exposing sites on the alpha subunit. Further, incubation of the insulin receptors with the combination of DTT and insulin also demonstrated the apparent insulin-potentiated subunit reduction without any increase in the total amount of [3H]NEM labeling.(ABSTRACT TRUNCATED AT 250 WORDS)

```
=> e longo m/au
E1          18      LONGO LUIGIA/AU
E2           6      LONGO LUIZ S JR/AU
E3         724 --> LONGO M/AU
E4         115      LONGO M A/AU
E5           9      LONGO M B/AU
E6          23      LONGO M C/AU
E7           2      LONGO M CASTELLANI/AU
E8          15      LONGO M D/AU
E9           2      LONGO M D C/AU
E10         20      LONGO M F/AU
E11         20      LONGO M G/AU
E12         15      LONGO M I/AU
```

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=> s e3
L7          724 "LONGO M"/AU

=> e flynn e/au
E1            1     FLYNN DUANE J/AU
E2            3     FLYNN DUSTIE/AU
E3          289 --> FLYNN E/AU
E4           56     FLYNN E A/AU
E5            2     FLYNN E B/AU
E6            5     FLYNN E C/AU
E7            9     FLYNN E D/AU
E8            3     FLYNN E E/AU
E9            1     FLYNN E F/AU
E10          11     FLYNN E H/AU
E11         152     FLYNN E J/AU
E12           1     FLYNN E J */AU

=> s e3
L8          289 "FLYNN E"/AU

=> s l7 or l8
L9         1012 L7 OR L8

=> d his

     (FILE 'HOME' ENTERED AT 14:13:20 ON 19 SEP 2006)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 14:13:50 ON 19 SEP 2006
L1         27165 S THIOREDOXIN
L2          1360 S (TRUNCATED OR MODIFIED) AND L1
L3       1932122 S STAIN? OR DYE?
L4            61 S L2 AND L3
L5             2 S (INCLUSION (W)BOD? ) AND L4
L6            27 DUP REM L4 (34 DUPLICATES REMOVED)
                 E LONGO M/AU
L7           724 S E3
                 E FLYNN E/AU
L8           289 S E3
L9          1012 S L7 OR L8

=> s l1 and l9
L10           2 L1 AND L9

=> dup rem l10
PROCESSING COMPLETED FOR L10
L11           2 DUP REM L10 (0 DUPLICATES REMOVED)

=> d 1-2 ibib ab

L11   ANSWER 1 OF 2  BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1998-09126  BIOTECHDS
TITLE:            Production of polypeptides as inclusion bodies;
                     recombinant protein preparation by plasmid pTrc99A or
                     plasmid pTrxfus vector-mediated thioredoxin
                     expression in Escherichia coli inclusion body
AUTHOR:           Chatterjee D; Longo M; Flynn E;
                  Oberfelder R
PATENT ASSIGNEE:  Life-Technol.
LOCATION:         Rockville, MD, USA.
PATENT INFO:      WO 9830684 16 Jul 1998
APPLICATION INFO: WO 1998-US492 8 Jan 1998
PRIORITY INFO:    US 1997-34658 8 Jan 1997
DOCUMENT TYPE:    Patent
```

LANGUAGE: English
OTHER SOURCE: WPI: 1998-399134 [34]
AB A new method for the preparation of a protein in the form of an inclusion body involves: obtaining a host cell (e.g. Escherichia coli) containing a first DNA sequence encoding the protein linked to a second DNA sequence encoding an inclusion partner (thioredoxin or modified thioredoxin), forming a gene fusion construct (plasmid pTrcpr1-monomer or pTrxA-concat); and culturing the cell to favor production of the protein as inclusion bodies. Also new are: vector plasmid pTrc99A and plasmid pTrxfus containing the construct; a host cell containing the vector; making a protein mol.weight ladder composition by obtaining one or more DNA sequences encoding proteins of different mol.weight values, transforming host cells with the DNA, culturing the cells to favor production of each protein, and isolating each protein; and making one or more stained proteins by incubating the proteins with one or more protein-binding dyes under incubation conditions to complex the proteins with the dyes. The methods may be used to prepare a fragment of the gene-32 protein of phage T4, a fragment of KpnI-methylase or a fragment of E. coli Dead-Box protein or thioredoxin. (84pp)

L11 ANSWER 2 OF 2 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 1997:421271 BIOSIS
DOCUMENT NUMBER: PREV199799720474
TITLE: Protein expression by inclusion.
AUTHOR(S): Oberfelder, R. W.; Flynn, E.; Chatterjee, D.
SOURCE: FASEB Journal, (1997) Vol. 11, No. 9, pp. A1200.
Meeting Info.: 17th International Congress of Biochemistry and Molecular Biology in conjunction with the Annual Meeting of the American Society for Biochemistry and Molecular Biology. San Francisco, California, USA. August 24-29, 1997.
CODEN: FAJOEC. ISSN: 0892-6638.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Oct 1997
Last Updated on STN: 8 Oct 1997

=> d his

(FILE 'HOME' ENTERED AT 14:13:20 ON 19 SEP 2006)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:13:50 ON 19 SEP 2006

L1	27165	S	THIOREDOXIN
L2	1360	S	(TRUNCATED OR MODIFIED) AND L1
L3	1932122	S	STAIN? OR DYE?
L4	61	S	L2 AND L3
L5	2	S	(INCLUSION (W)BOD?) AND L4
L6	27	DUP REM	L4 (34 DUPLICATES REMOVED)
		E	LONGO M/AU
L7	724	S	E3
		E	FLYNN E/AU
L8	289	S	E3
L9	1012	S	L7 OR L8
L10	2	S	L1 AND L9
L11	2	DUP REM	L10 (0 DUPLICATES REMOVED)

	L #	Hits	Search Text
1	L1	6428	thioredoxin
2	L2	1162132	truncta$3 or modified
3	L3	825	l1 same l2
4	L4	426282	stain$3 or dy$2
5	L5	10	l3 same l4
6	L6	397864	molecular adj weight
7	L7	7	l3 same l6
8	L8	8117	inclusion adj bod$3
9	L9	34	l3 same l8
10	L10	34576	CHATTERJEE LONGO FLYNN
11	L11	1	l5 and l10
12	L12	27	l3 and l10

	Issue Date	Pages	Document ID	Title
1	20040610	72	US 20040109875 A1	Pro-apoptotic bacterial vaccines to enhance cellular immune responses

	Issue Date	Pages	Document ID	Title
1	20060831	127	US 20060194275 A1	Transporter and ion channels
2	20060615	59	US 20060128660 A1	FK228 analogs and methods of making and using the same
3	20060216	83	US 20060035315 A1	Transporters and ion channels
4	20041209	144	US 20040248251 A1	Receptors and membrane associated proteins
5	20041111	171	US 20040224911 A1	Transporters and ion channels
6	20041014	31	US 20040204563 A1	Methods for production of proteins
7	20040805	72	US 20040152874 A1	Transporter and ion channels
8	20040701	46	US 20040127683 A1	Transporters and ion channels
9	20040617	140	US 20040116666 A1	Transporters and ion channels
10	20040610	72	US 20040109875 A1	Pro-apoptotic bacterial vaccines to enhance cellular immune responses
11	20040318	182	US 20040053258 A1	Transporters and ion channels
12	20040205	140	US 20040024183 A1	Transporters and ion channels
13	20040122	119	US 20040014945 A1	Transporters and ion channels
14	20031120	144	US 20030216310 A1	Transporters and ion channels
15	20031113	94	US 20030211499 A1	Transporters and ion channels

	Issue Date	Pages	Document ID	Title
16	20030911	121	US 20030171275 A1	Transporters and ion channels
17	20030724	616	US 20030138890 A1	Novel G protein-coupled receptor family members, human thioredoxin family members, human leucine-rich repeat family members, and human ringfinger family member
18	20030501	29	US 20030083234 A1	Anti-angiogenesis methods, compositions and uses therefor
19	20030313	64	US 20030049700 A1	22108 and 47916, novel human thioredoxin family members and uses thereof
20	20020530	35	US 20020065392 A1	METHODS FOR PRODUCTION OF PROTEIN
21	20020321	50	US 20020034801 A1	22105, a novel human thioredoxin family member and uses thereof
22	20040309	29	US 6703484 B2	Methods for production of proteins
23	20031125	30	US 6653068 B2	Generation of specific binding partners binding to (poly)peptides encoded by genomic DNA fragments or ESTs
24	20021008	46	US 6462187 B1	22109, a novel human thioredoxin family member and uses thereof

	Issue Date	Pages	Document ID	Title
25	20010320	48	US 6204248 B1	Pharmaceutical preparations of glutathione and methods of administration thereof
26	19991012	19	US 5965399 A	Cloning and expression of rat liver and porcine liver ribonuclease inhibitor
27	19990803	34	US 5932440 A	Mammalian ribonuclease inhibitors and use thereof